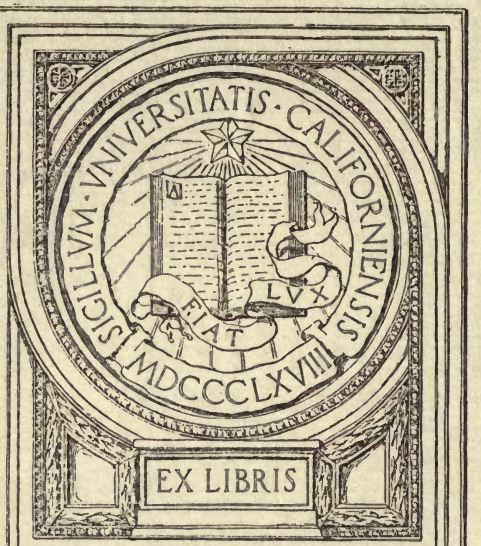

Some Characteristics of Invertase Action and their Significance in Interpreting the Nature of the Reaction.

DISSERTATION

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN THE FACULTY OF PURE SCIENCE, COLUMBIA UNIVERSITY.

By

GROVER BLOOMFIELD

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TO MY PARENTS AND C. L. B.

ACKNOWLEDGEMENT.

The author wishes to express his sincere gratitude to Professor John M. Nelson for his direction of this work which has always been an inspiration and incentive to greater efforts.

Thanks are also due to the Harriman Research Laboratory, New York City, whose financial assistance made this work possible.

Historical. Brown (J. Chem. Soc., 81, 373 [1902]) observed that the rate of hydrolysis of sucrose in the presence of invertase reaches a maximum and remains constant above a certain substrate concentration, but did not actually determine what this concentration was.

Nelson and Vosburgh (J. Am. Chem. Soc., 39, 790 [1919]) discussed a number of curves obtained from their own data and those of Michaelis and Menten (Biochem. Z., 49, 333 [1913]) relating the velocity of hydrolysis with the sucrose concentration and showed that the maximum rate of inversion was obtained in each case at about five grams of substrate per 100 c. c. of solution. At higher concentrations there was practically no change in velocity, while with less sugar the rate was smaller.

The fact that two distinct invertase preparations, used at different temperatures (Nelson and Vosburgh worked at 37°, Michaelis and Menten at 25°), although causing different actual rates of hydrolysis, gave a maximum velocity of inversion at identical sucrose concentrations, is very striking for it indicates that there is some sort of phenomenon which is independent of the enzyme preparation and the temperature.

The Relation Between the Sucrose Concentration and the Rate of Hydrolysis at Various Temperatures and Hydrogen Ion Concentrations.

The fact that the temperature does not seem to affect the sucrose concentration at which the hydrolysis of the substrate reaches a maximum, but does affect the actual rate of the reaction appeared so striking that it was deemed desireable to obtain further data on this point. It was hoped that more light would thus be thrown upon the nature of the hydrolytic process.

Hence, the influence of the temperature upon the relation between the sucrose concentration and the rate of the hydrolysis and, in addition to this, the influence of the hydrogen ion concentration were studied.

Experimental. Curves were obtained in such ranges of temperature and hydrogen ion concentration (see Tables I—IV) as to make the results significant for the subsequent part of this investigation.

Since changes of the hydrogen ion concentration and temperature affect the velocity of hydrolysis, the curves obtained by plotting the latter against the sucrose concentration would,

naturally, not be comparable. In order to make a comparison possible (and this was the object of the following experiments), the velocities all had to be reduced to a single scale. This was done by giving the value of unity to the maximum velocity for each curve, so that the lower velocities in each case would become fractions of the maximum.

The relative velocities of hydrolysis were obtained by Michaelis and Menten's method (loc. cit.) of comparing the initial rates of inversion. If, at the beginning of the reaction, the amounts of sucrose hydrolysed are plotted against the corresponding times, practically straight lines are obtained. The slopes of these initial straight line sections were assumed to give the relative velocities.

This method of comparing the rates of hydrolysis at the beginning of the reaction rather than at some later stage, as was done by Nelson and Vosburgh, has the advantage that in the former case practically no invert sugar is present, while in the latter sufficient invert sugar may have formed to introduce a complicating factor due to its retarding influence on the hydrolysis.

Dependecy of the Velocity of Hydrolysis on the Sucrose Concentration at Various Temperatures and Hydrogen ion Concentrations.

TABLE I. Temperature 15°.

$p_H = 4.7$

Experiment No. F1. 10 g. Sucrose per 100 cc.

Time minutes	Observed rotation degrees	Change rotation degrees
0	13.03 (extr.)	—
2	12.84	0.19
4	12.65	0.38
8	12.26	0.77
12	11.89	1.14
16	11.52	1.51

Experiment No. F2 5 g. Sucrose per 100 cc.

Time minutes	Observed rotation degrees	Change rotation degrees
0	6.51 (extr.)	—
2	6.32	0.19
4	6.12	0.39
8	5.74	0.77
12	5.39	1.12
16	5.02	1.49

Experiment No. F3. 4 g. Sucrose per 100 cc.

Time minutes	Observed rotation degrees	Change rotation degrees
0	5.225 (extr.)	—
2	5.04	0.185
4	4.85	0.375
7	4.57	0.655
10	4.29	0.935
14	3.92	1.305

Experiment No. F4. 2.5 g Sucrose. per 100 cc.

Time minutes	Observed rotation degrees	Change rotation degrees
0	3.29 (extr.)	—
2	3.12	0.17
4	2.95	0.34
7	2.69	0.60
10	2.45	0.84
14	2.14	1.15

Experiment No. F 5. 1.875 g. Sucrose per 100 cc.

Time minutes	Observed rotation degrees	Change rotation degrees
0	2.48 (extr.)	—
2	2.32	0.16
4	2.16	0.32
7	1.93	0.55
10	1.71	0.77
14	1.43	1.05

Experiment No. F 6. 1.25 g. Sucrose per 100 cc.

Time minutes	Observed rotation degrees	Change rotation degrees
0	1.69 (extr.)	—
2	1.55	0.14
4	1.42	0.27
7	1.20	0.49
10	1.01	0.68
14	0.81	0.88

Experiment No. F 7. 0.625 g. Sucrose per 100 cc.

Time minutes	Observed rotation degrees	Change rotation degrees
0	0.85 (extr.)	—
2	0.76	0.09
4	0.67	0.18
7	0.54	0.31
10	0.43	0.42
14	0.30	0.55

TABLE II. Temperature 25°.

(A). $p_H = 3.25$.

Experiment No. A 1. 12 g. Sucrose per 100 cc.

Time minutes	Observed rotation degrees	Change rotation degrees
0	15.62 (extr.)	—
1.5	15.43	0.19
3.0	15.23	0.39
4.5	15.03	0.59
7.0	14.70	0.92
10.0	14.30	1.32
13.0	13.92	1.70

Experiment No. A 2. 10 g. Sucrose per 100 cc.

Time minutes	Observed rotation degrees	Change rotation degrees
0	13.04 (extr.)	—
1.5	12.84	0.20
3.0	12.64	0.40
4.5	12.44	0.60
7.0	12.12	0.92
10.0	11.70	1.34
13.0	11.31	1.73

Experiment No. A 3. 5 g. Sucrose per 100 cc.

Time minutes	Observed rotation degrees	Change rotation degrees
0	6.54 (extr.)	—
1.5	6.35	0.19
3.0	6.15	0.39
4.5	5.95	0.59
7.0	5.62	0.92
10.0	5.22	1.32
13.0	4.84	1.70

Experiment No. A 4. 2.5 g. Sucrose per 100 cc.

Time minutes	Observed rotation degrees	Change rotation degrees
0	3.27 (extr.)	—
1.5	3.10	0.17
3.0	2.92	0.35
4.5	2.75	0.52
7.0	2.45	0.82
10.0	2.11	1.16
13.0	1.77	1.50

Experiment No. A5. 1.875 g. Sucrose per 100 cc.

Time minutes	Observed rotation degrees	Change rotation degrees
0	2.41 (extr.)	—
1.5	2.25	0.16
3.0	2.10	0.31
4.5	1.93	0.48
7.0	1.66	0.75
10.0	1.37	1.04
13.0	1.08	1.33

Experiment No. A6. 1.25 g. Sucrose per 100 cc.

Time minutes	Observed rotation degrees	Change rotation degrees
0	1.58 (extr.)	—
1.5	1.45	0.13
3.0	1.33	0.25
4.5	1.20	0.38
7.0	1.00	0.58
10.0	0.79	0.79
13.0	0.61	0.97

Experiment No. A7. 0.625 g. Sucrose per 100 cc.

Time minutes	Observed rotation degrees	Change rotation degrees
0	0.81 (extr.)	—
1.5	0.73	0.08
3.0	0.65	0.16
4.5	0.56	0.25
7.0	0.45	0.36
10.0	0.34	0.47
13.0	0.22	0.59

(B). $p_H = 4.7$.

Experiment No. B1. 12 g. Sucrose per 100 cc.

Time minutes	Observed rotation degrees	Change rotation degrees
0	15.64 (extr.)	—
1	15.47	0.17
2	15.30	0.34
3	15.12	0.52
5	14.80	0.84
7	14.49	1.15
9	14.18	1.46

Experiment No. B2. 10 g. Sucrose per 100 cc.

Time minutes	Observed rotation degrees	Change rotation degrees
0	13.02 (extr.)	—
1	12.85	0.17
2	12.71	0.33
3	12.52	0.50
5	12.19	0.83
7	11.85	1.17
9	11.55	1.47

Experiment No. B3. 5 g. Sucrose per 100 cc.

Time minutes	Observed rotation degrees	Change rotation degrees
0	6.56 (extr.)	—
1	6.40	0.16
2	6.25	0.31
3	6.07	0.49
5	5.77	0.79
7	5.41	1.15
9	5.12	1.44

Experiment No. B4. 2.5 g. Sucrose per 100 cc.

Time minutes	Observed rotation degrees	Change rotation degrees
0	3.32 (extr.)	—
1	3.17	0.15
2	3.02	0.30
3	2.85	0.47
5	2.55	0.77
7	2.28	1.04
9	2.02	1.30

Experiment No. B5. 1.875 g. Sucrose per 100 cc.

Time minutes	Observed rotation degrees	Change rotation degrees
0	2.49 (extr.)	—
1	2.35	0.14
2	2.21	0.28
3	2.08	0.41
5	1.81	0.68
7	1.58	0.91
9	1.37	1.12

Experiment No. B6. 1.25 g. Sucrose per 100 cc.

Time minutes	Observed rotation degrees	Change rotation degrees
0	1.65 (extr.)	—
1	1.54	0.11
2	1.43	0.22
3	1.31	0.34
5	1.10	0.55
7	0.93	0.72
9	0.78	0.87

Experiment No. B7. 0.625 g. Sucrose per 100 cc.

Time minutes	Observed rotation degrees	Change rotation degrees
0	0.805 (extr.)	—
1	0.73	0.075
2	0.66	0.145
3	0.58	0.225
5	0.45	0.355
7	0.32	0.485
9	0.24	0.565

(C) $p_{H^{\cdot}} = 6.67$.

Experiment No. C1. 12 g. Sucrose per 100 cc.

Time minutes	Observed rotation degrees	Change rotation degrees
0	15.64 (extr.)	—
1.5	15.46	0.18
3.0	15.28	0.36
4.5	15.10	0.54
7.0	14.85	0.79
10.0	14.52	1.12
13.0	14.17	1.47

Experiment No. C2. 10 g. Sucrose per 100 cc.

Time minutes	Observed rotation degrees	Change rotation degrees
0	13.06 (extr.)	—
1.5	12.88	0.18
3.0	12.71	0.35
4.5	12.53	0.53
7.0	12.27	0.79
10.0	11.95	1.11
13.0	11.59	1.47

Experiment No. C3. 5 g. Sucrose per 100 cc.

Time minutes	Observed rotation degrees	Change rotation degrees
0	6.52 (extr.)	—
1.5	6.35	0.17
3.0	6.16	0.36
4.5	5.99	0.53
7.0	5.74	0.78
10.0	5.34	1.11
13.0	5.07	1.45

Experiment No. C4. 2.5 g. Sucrose per 100 cc.

Time minutes	Observed rotation degrees	Change rotation degrees
0	3.30 (extr.)	—
1.5	3.14	0.16
3.0	2.98	0.32
4.5	2.83	0.47
7.0	2.61	0.69
10.0	2.31	0.99
13.0	2.02	1.28

Experiment No. C5. 1.875 g. Sucrose per 100 cc.

Time minutes	Observed rotation degrees	Change rotation degrees
0	2.52 (extr.)	—
1.5	2.37	0.15
3.0	2.22	0.30
4.5	2.07	0.45
7.0	1.84	0.68
10.0	1.58	0.94
13.0	1.36	1.16

Experiment No. C6. 1.25 g. Sucrose per 100 cc.

Time minutes	Observed rotation degrees	Change rotation degrees
0	1.62 (extr.)	—
1.5	1.49	0.13
3.0	1.37	0.25
4.5	1.23	0.39
8.0	0.98	0.64
10.0	0.82	0.80
13.0	0.58	1.04

Experiment No. C7. 0.625 g. Sucrose per 100 cc.

Time minutes	Observed rotation degrees	Change rotation degrees
0	0.81 (extr.)	—
1.5	0.73	0.08
3.0	0.65	0.16
4.5	0.56	0.25
7.0	0.39	0.39
10.0	0.27	0.54
13.0	0.16	0.65

TABLE III. Temperature 30°.

$p_{H^{\cdot}} = 4.7$.

Experiment No. D1. 12 g. Sucrose per 100 cc.

Time minutes	Observed rotation degrees	Change rotation degrees
0	15.70 (extr.)	—
1	15.50	0.20
2	15.30	0.40
3	15.09	0.61
5	14.69	1.01
7	14.27	1.43
9	13.86	1.84

Experiment No. D2. 10 g. Sucrose per 100 cc.

Time minutes	Observed rotation degrees	Change rotation degrees
0	13 12 (extr.)	—
1	12.92	0.20
2	12.72	0.40
3	12.51	0.61
5	12.09	1.03
7	11.68	1.44
9	11.29	1.83

Experiment No. D3. 5 g. Sucrose per 100 cc.

Time minutes	Observed rotation degrees	Change rotation degrees
0	6.50 (extr.)	—
1	6.31	0.19
2	6.12	0.38
3	5.91	0.59
5	5.51	0.99
7	5.11	1.39
9	4.73	1.77

Experiment No. D4. 2.5 g. Sucrose per 100 cc.

Time minutes	Observed rotation degrees	Change rotation degrees
0	3.285 (extr.)	—
1	3.11	0.175
2	2.94	0.345
3	2.75	0.535
5	2.40	0.885
7	2.05	1.235
9	1.72	1.565

Experiment No. D 5. 1.875 g. Sucrose per 100 cc.

Time minutes	Observed rotation degrees	Change rotation degrees
0	2.42 (extr.)	—
1	2.27	0.15
2	2.12	0.30
3	1.95	0.47
5	1.64	0.78
7	1.35	1.07
9	1.09	1.33

Experiment No. D 6. 1.25 g. Sucrose per 100 cc.

Time minutes	Observed rotation degrees	Change rotation degrees
0	1.64 (extr.)	—
1	1.50	0.14
2	1.37	0.27
3	1.23	0.41
5	0.96	0.68
7	0.71	0.93
9	0.51	1.13

Experiment No. D 7. 0.625 g. Sucrose per 100 cc.

Time minutes	Observed rotation degrees	Change rotation degrees
0	0.83 (extr.)	—
1	0.74	0.09
2	0.65	0.18
3	0.55	0.28
5	0.38	0.45
7	0.25	0.58
9	0.18	0.65

TABLE IV. Temperature 35°.

$p_H = 4.7$

Experiment No. E 1. 10 g. Sucrose per 100 cc.

Time minutes	Observed rotation degrees	Change rotation degrees
0	12.97 (extr.)	—
1	12.72	0.25
2	12.48	0.49
5	11.72	1.25
8	10.96	2.01
15	9.71	3.26
19	8.23	4.74

Experiment No. E 2. 5 g. Sucrose per 100 cc.

Time minutes	Observed rotation degrees	Change rotation degrees
0	6.55 (extr.)	—
1	6.31	0.24
2	6.07	0.48
5	5.32	1.23
9	4.33	2.22
11	3.87	2.68
14	3.22	3.33

Experiment No. E 3. 2.5 g. Sucrose per 100 cc.

Time minutes	Observed rotation degrees	Change rotation degrees
0	3.27 (extr.)	—
1	3.05	0.22
2	2.83	0.44
4	2.39	0.88
6	1.95	1.32
9	1.38	1.85
13	0.76	2.51

Experiment No. E 4. 1.875 g. Sucrose per 100 cc.

Time minutes	Observed rotation degrees	Change rotation degrees
0	2.41 (extr.)	—
1	2.21	0.20
2	2.01	0.40
4	1.62	0.79
6	1.26	1.15
9	0.85	1.56
13	0.32	2.09

Experiment No. E5. 1.25 g. Sucrose per 100 cc.

Time minutes	Observed rotation degrees	Change rotation degrees
0	1.65 (extr.)	—
1	1.48	0.17
2	1.31	0.34
4	0.97	0.68
6	0.72	0.93
9	0.41	1.24
13	0.02	1.63

Experiment No. E6. 0.625 g. Sucrose per 100 cc.

Time minutes	Observed rotation degrees	Change rotation degrees
0	0.81 (extr.)	—
1	0.71	0.10
2	0.60	0.21
3	0.50	0.31
4	0.42	0.39
5	0.33	0.48
8	0.12	0.69

From Tables I, II, III and IV, time-change of rotation curves were plotted and the initial velocities compared for each set of experiments. The greatest velocities were always taken as unity while the others were given in fractions thereof. In this way all data were reduced to one scale. The relative velocities thus obtained are given in Table V and plotted in Figure 1.

TABLE V.

Relation of Velocity of Hydrolysis to the Sucrose Concentration for Varying Conditions of Hydrogen ion Concentration and Temperature.

g Sucrose per 100 cc.	Relative Velocities					
	Exp. F	Exp. A	Exp. B	Exp. C	Exp. D	Exp. E
12	1.000	1.000	1.000	1.000	—	—
10	1.000	1.000	1.000	1.000	1.000	1.000
5	1.000	1.000	0.985	0.977	0.972	1.000
2.5	0.889	0.894	0.924	0.878	0.872	0.870
1.875	0.813	0.818	0.821	0.823	0.778	0.792
1.25	0.707	0.654	0.680	0.693	0.677	0.677
0.625	0.448	0.425	0.445	0.459	0.450	0.411

The points of the different sets of experiments, as is seen in Figure 1, show no consistent shifting due to changes of temperature and hydrogen-ion concentration, and fall on a practically smooth curve. This shows that the temperature and hydrogen-ion concentration do not affect the relation between the sucrose concentration and the relative rates of the hydrolysis; or stated differently, that the effect of the temperature and hydrogen ion concentration on the velocity of the reaction is independent of the sucrose concentration.

Furthermore, the results show that the sucrose concentration at which the hydrolysis reaches its maximum velocity is independent of the temperature and the hydrogen ion concentration.

When the actual velocities in above experiments, at the three hydrogen ion concentrations at 25°, were plotted instead of the relative velocities, the curves shown in Figure 2 were obtained.

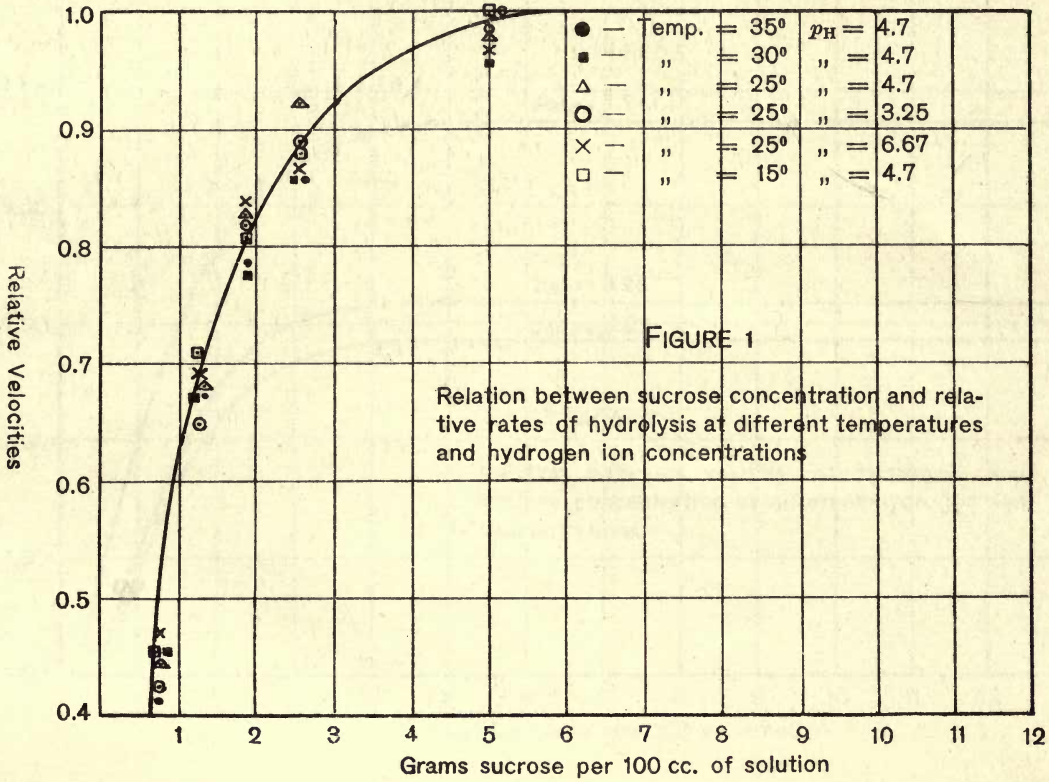
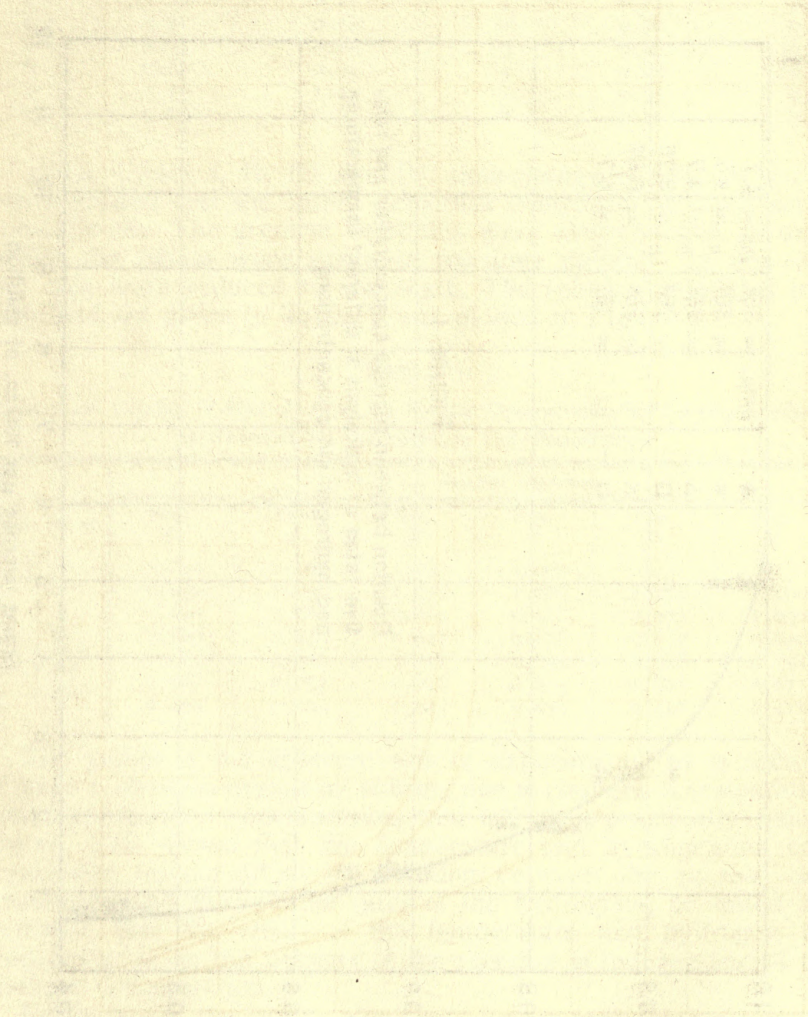


FIGURE 1

Relation between sucrose concentration and relative rates of hydrolysis at different temperatures and hydrogen ion concentrations



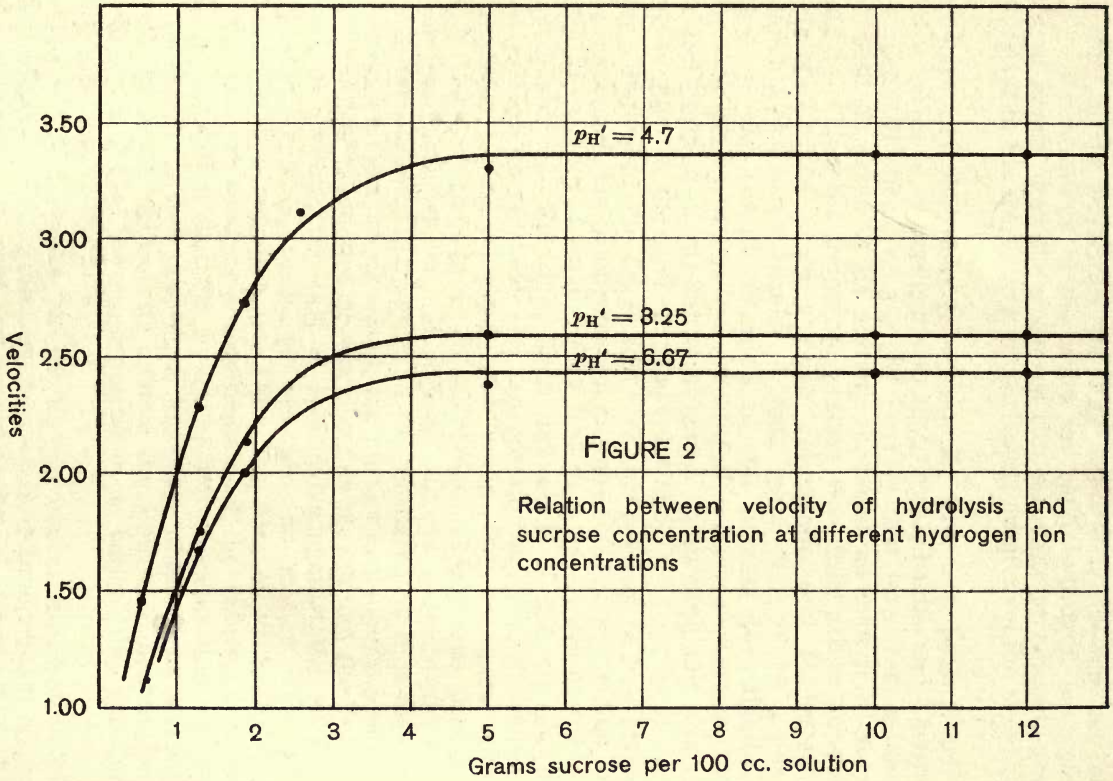
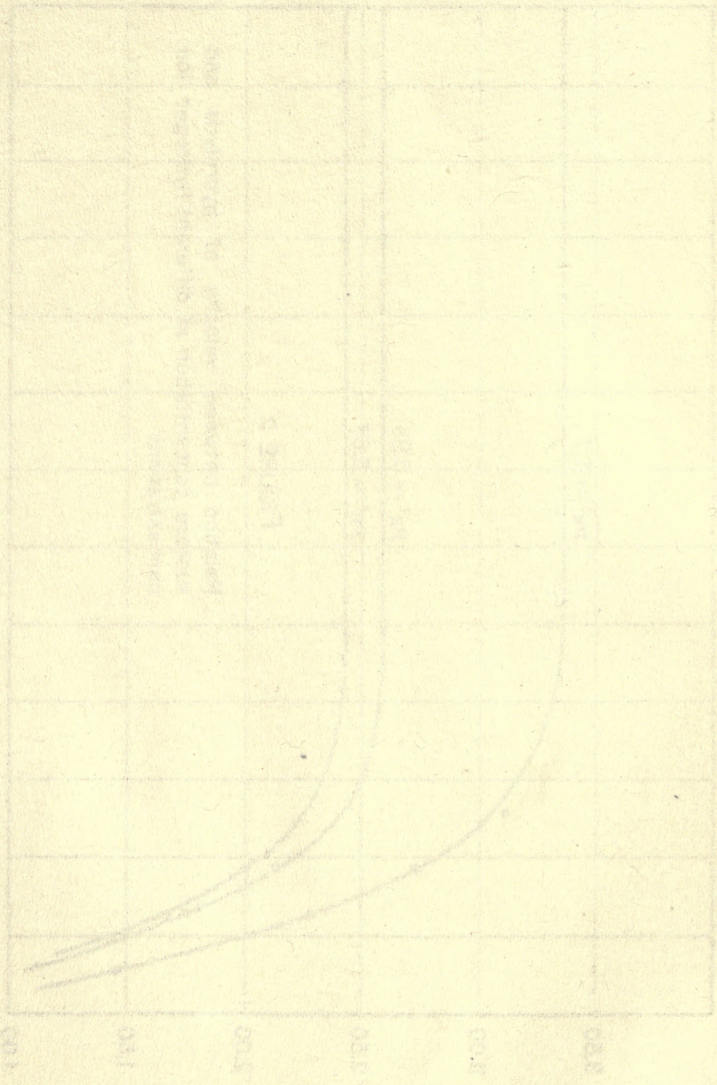


FIGURE 2

Relation between velocity of hydrolysis and sucrose concentration at different hydrogen ion concentrations



The fact that these curves could be made to coincide (within the limits of the experimental error) by changing the ordinate scale means that the ratio of the velocities for any arbitrarily chosen set of sucrose concentrations (say 2.5, 5 and 10 grams per 100 cc. of solution) is the same for each curve, or what amounts to the same thing, for each hydrogen-ion concentration. Conversely, if the ratio of the velocities for any arbitrarily chosen set of sucrose concentrations is the same for each hydrogen ion concentration curve, then these curves can be made to coincide (as was the case in Figure 1) by a proper transformation of the velocity scale.

Michaelis and Rothstein (Biochem. Z., 110, 217 [1920]) performed a series of experiments to test out Michaelis and Davidsohn's idea (Biochem. Z. 35, 387 [1911]) that invertase, in the more alkaline region of its activity, behaved as though it were an acid in which the unionized portion was responsible for the catalytic properties of the enzyme. Michaelis and Rothstein's experiments, however, furnished data which serves as a confirmation of the results obtained in the present investigation, namely that the hydrogen ion concentration has no effect on the shape and position of the curve obtained by plotting the rate of hydrolysis against the sucrose concentration.

They determined the dependency of the velocity of hydrolysis on the hydrogen ion concentration, using a different amount of sucrose for each set of experiments. The data gave a set of curves similar in shape to the right hand branches of the curves in Figure 3, each curve, however, representing a different sucrose concentration. They claimed that when the ordinates were properly reduced, the curves all coincided. This means that the ratio of the velocities for the various sucrose concentrations is the same for any one hydrogen ion concentration as it is for any other since, as the authors stated, the curves are superimposable. But, as was just mentioned above, this fact means that the curves obtained by plotting the rate of hydrolysis against the sucrose concentration can be made to coincide, no matter what the hydrogen ion concentration is. Thus, the data of Michaelis and Rothstein can be used as a confirmation of the results obtained in this investigation that the hydrogen ion concentration has no effect on the shape and position of the curve obtained by plotting the velocity of the hydrolysis against the sucrose concentration, and hence on the sucrose concentration at which the reaction reaches a maximum velocity.

In view of above results that the sucrose concentration at which the hydrolysis reaches a maximum velocity is independent of the temperature and hydrogen ion concentration, it follows that the influence of these two factors must be ascribed to that part of the

process which appears to be more intimately related to the actual catalytic effect of the enzyme.

The Influence of the Temperature and Hydrogen ion Concentration on the Catalytic Effect of the Enzyme.

This influence was studied as follows: Curves relating the hydrogen ion concentration to the rate of hydrolysis were obtained at three temperatures in order to study:

1. The effect of the hydrogen ion concentration on the velocity of the reaction, in order to see what the shape of the curves was and whether it was affected by changes of temperature.

2. The temperature coefficient of the reaction at different hydrogen ion concentrations.

A Criterion for the Rate of Hydrolysis. Nelson and Vosburgh (loc. cit.) have definitely established that the hydrolysis of sucrose in the presence of invertase does not follow the unimolecular law. The constant unimolecular coefficients obtained by Hudson (J. Am. Chem. Soc., 30, 1160; 1564 [1908]) must have been due to special conditions holding for his particular experiments.

Nelson and Hitchcock (J. Am. Chem. Soc., 43, 2632 [1921]) derived an expression for the rate of hydrolysis which is given by

$$n = \frac{1}{t}\left(\log \frac{100}{100 - p} + 0.002642\,p - 0.0_5886\,p^2 - 0.0_6 1034\,p^3\right).$$

n, which remains constant throughout any one hydrolysis (if the latter is not deviated from its normal course by the inactivation of the enzyme, or some other reason), is a measure of the velocity of the reaction, and p is the percentage of sucrose hydrolysed in time t.

This expression, as the authors have pointed out, is purely empirical. It was intended to be used in such hydrolyses only in which the sucrose concentration was 10 grams per 100 cc. of solution, and was shown to hold in the temperature interval 15° to 35° and between $p_H = 4.5$ and 6.5.

Experimental. The experiments in Tables VI, VII and VIII were performed with above initial sucrose concentration and 5.56 cc. invertase per 100 cc. of solution throughout. The hydrogen ion concentrations were maintained constant with 0.01 molar citrate buffers, except in a few cases where, as indicated, borate buffers were used. The progress of the hydrolyses was followed by the polariscopic method which Vosburgh (J. Am. Chem. Soc., 43, 219 [1921]) has shown to be applicable. The invertase preparation numbered 7 in this laboratory was used for all experiments.

Above temperatures were chosen because much of the previous work had been done in this range and a means was thus given for fruitful comparison. Besides, practical considerations made

working at lower and higher temperatures inadvisable. Below 25° the rate of hydrolysis was so slow that the time of the experiments became unduly long unless the amount of invertase was increased, and this was undesirable for practical considerations. Above 35° the range of constant n-values was unduly reduced due to the inactivation of the enzyme in the more acid region of its activity.

The temperature intervals were large enough for well-defined

Velocity of Hydrolysis at Various Temperatures and Hydrogen ion Concentrations.

TABLE VI. Temperature 25°.

Experiment No. 46. $p_H' = 8.34$ Borate.

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$
0	13.05	—	—
60	12.91	0.8312	10
120	12.77	1.663	10
180	12.62	2.554	10
240	12.47	3.443	10
		Mean	10

Experiment No. 45. $p_H' = 7.70$.

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$
0	13.04	—	—
60	11.43	9.559	113
180	8.27	28.32	116
248	6.48	38.95	120
335	4.28	52.01	125
		First Value	113

Experiment No. 47. $p_H' = 7.43$.

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$
0	13.06	—	-
105	8.42	27.55	194
150	6.34	37.89	192
215	4.35	51.71	193
		Mean	193

Experiment No. 10. $p_H' = 7.33$.

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$
0	13.05	—	—
57	10.22	16.84	213
105	8.01	29.97	213
143	6.37	39.74	213
191	4.46	51.10	214
∞	—3.76	— Mean	213

Experiment No. 11. $p_H' = 6.92$.

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$
0	13.05	—	—
55	8.36	27.92	376
77	6.69	37.86	374
105	4.74	49.46	374
137	2.79	61.06	374
∞	—3.75	— Mean	375

Experiment No. 48. $p_H' = 6.67$.

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$
0	13.05	—	—
45	8.18	28.79	475
63	6.47	39.06	474
81	4.90	48.40	473
102	3.24	58.25	473
		Mean	474

Experiment No. 13. $p_{H'} = 6.48$.

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$
0	13.07	—	—
43	8.05	29.80	516
61	6.19	40.83	515
79	4.54	50.56	511
97	3.05	59.32	510
∞	— 3.78	—	
		Mean	514

Experiment No. 8. $p_{H'} = 6.25$.

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$
0	13.03	—	—
37	8.24	28.58	573
51	6.62	38.24	572
69	4.73	49.52	570
90	2.79	61.09	572
∞	— 3.73	—	
		Mean	572

Experiment No. 12. $p_{H'} = 5.67$.

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$
0	13.11	—	—
34	8.06	29.95	656
47	6.35	40.09	654
62	4.57	50.65	652
78	2.90	60.54	652
		Mean	654

Experiment No. 17. $p_{H'} = 5.32$.

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$
0	13.02	—	—
33	7.96	30.07	679
45	6.33	39.75	677
59	4.58	50.14	677
75	2.83	60.52	678
∞	— 3.81	—	
		Mean	678

Experiment No. 40. $p_{H'} = 4.88$.

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$
0	13.05	—	—
33	7.96	30.22	682
45	6.31	40.05	682
59	4.56	50.44	682
75	2.81	60.84	682
		Mean	682

Experiment No. 41. $p_{H'} = 4.59$.

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$
0	13.05	—	—
33	7.96	30.22	682
45	6.31	40.05	682
59	4.58	50.29	679
75	2.81	60.84	682
		Mean	681

Experiment No. 42. $p_{H'} = 4.27$.

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$
0	13.05	—	—
33	8.03	29.81	672
45	6.40	39.49	672
59	4.68	49.69	670
75	2.93	60.08	671
		Mean	671

Experiment No. 27. $p_{H'} = 4.06$.

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$
0	13.06	—	—
33	8.09	29.47	664
45	6.50	38.90	661
59	4.80	48.99	658
75	3.10	59.12	660
		Mean	660

Experiment No. 44. Borate. $p_{H'} = 4.02$.

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$
0	13.05	—	—
45	6.56	38.53	653
57	5.06	47.43	655
67	3.96	53.98	653
77	2.93	60.08	653
		Mean	654

Experiment No. 43. Borate. $p_{H'} = 3.76$

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$
0	13.05	—	—
60	4.85	48.68	642
70	3.75	55.22	643
80	2.78	60.96	642
90	1.94	65.97	638
		Mean	641

Experiment No. 37. $p_{H'} = 3.68$.

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$
0	13.05	—	—
11.5	11.33	10.21	633
23	9.68	20.01	632
36	7.91	30.52	632
51	6.04	41.62	629
68	4.12	53.03	629

Mean 631

Experiment No. 24. $p_{H'} = 3.25$.

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$
0	13.03	—	—
57	5.76	43.19	587
73	4.07	53.23	589
83	3.17	58.59	586
93	2.31	63.69	587
∞	— 3.80	—	

Mean 587

Experiment No. 29. $p_{H'} = 2.85$.

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$
0	13.03	—	—
40	8.00	29.86	556
55	6.32	39.74	554
72	4.61	49.99	553
91	2.95	59.82	550

Mean 553

Experiment No. 34. $p_{H'} = 2.76$.

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$
0	13.06	—	—
13	11.40	9.856	540
27	9.74	19.71	530
42	8.08	29.56	524
59	6.35	39.84	517
78	4.59	50.29	514

Decreasing values of n, take the first.

Experiment No. 31. $p_{H'} = 2.37$.

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$
0	13.03	—	—
16	11.17	11.04	492
31	9.57	20.54	482
45	8.14	29.03	479
70	5.86	42.22	466

Decreasing values of n, take the first.

Experiment No. 39. $p_{H'} = 2.18$.

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$
0	13.05	—	—
17	11.22	10.86	455
34	9.77	19.47	416
56	8.00	29.98	399
79	6.37	39.67	385
110	4.44	51.12	372

Decreasing values of n, take the first.

TABLE VII. Temperature 30°.

Experiment No. 15 a. $p_{H'} = 8.41$. Borate.

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$
0	13.08	—	—
48	12.86	1.306	18
96	12.67	2.432	18
144	12.48	3.563	17
192	12.26	4.868	18

Mean 18

Experiment No. 13 a. $p_{H'} = 7.67$.

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$
0	13.05	—	—
120	8.46	27.26	168
180	6.35	39.78	169
240	4.47	50.93	170
310	2.48	62.75	172

Mean 170

Experiment No. 12 a. $p_{H'} = 7.45$.

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$
0	13.05	—	—
64	9.12	23.33	267
90	7.62	32.24	268
115	6.25	40.37	270
140	4.98	47.92	270

Mean 269

Experiment No. 11 a. $p_{H'} = 7.21$.

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$
0	13.07	—	—
47	9.14	23.33	364
75	7.00	36.04	364
120	4.01	53.79	363
140	2.88	60 47	363

Mean 364

Experiment No. 10a. $p_{H'} = 6.79$.

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$
0	13.05	—	—
37	8.00	29.98	603
50	6.43	39.31	602
66	4.63	49.99	603
82	3.07	59.26	602
		Mean	603

Experiment No. 14a. $p_{H'} = 6.52$.

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$
0	13.07	—	—
30	8.39	27.78	686
42	6.69	37.87	687
55	5.02	47.79	685
75	2.80	60.96	684
		Mean	686

Experiment No. 17a. $p_{H'} = 5.80$.

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$
0	13.07	—	—
27	8.03	29.92	825
38	6.21	40.72	824
49	4.55	50.58	822
61	2.91	60.20	827
		Mean	825

Experiment No. 7a. $p_{H'} = 5.29$.

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$
0	13.06	—	—
26	7.99	30.12	863
35	6.42	39.44	863
47	4.54	50.53	858
62	2.54	62.65	860
		Mean	861

Experiment No. 25a. $p_{H'} = 4.88$.

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$
0	13.07	—	—
26	7.96	30.34	870
35	6.38	39.72	869
46	4.60	50.29	871
58	2.93	60.20	870
		Mean	870

Experiment No. 19a. $p_{H'} = 4.68$.

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$
0	13.07	—	—
26	7.96	30.34	870
35	6.37	39.78	871
46	4.59	50.35	873
58	2.93	60.20	870
		Mean	870

Experiment No. 21a. $p_{H'} = 4.27$.

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$
0	13.08	—	—
26	8.04	29.92	857
35	6.48	39.18	856
46	4.75	49.45	854
58	3.09	59.32	853
		Mean	855

Experiment No. 22a. $p_{H'} = 3.66$.

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$
0	13.08	—	—
9	11.39	10.03	793
18	9.79	19.59	790
28	8.08	29.69	789
41	6.06	41.68	784
		Mean	789

Experiment No. 26a. $p_{H'} = 3.24$.

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$
0	13.05	—	—
10	11.30	10.39	739
20	9.63	20.31	739
31	7.90	30.58	736
42	6.27	40.25	736
55	4.52	50.64	735
		Mean	737

Experiment No. 23a. $p_{H'} = 2.93$.

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$
0	13.06	—	—
10.5	11.34	10.21	693
21	9.74	19.71	682
32	8.17	29.03	677
45	6.41	39.49	672
58	4.83	48.87	668

Decreasing values of n, take the first.

Experiment No. 24 a. $p_{H'} = 2.36$.

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$
0	13.07	—	—
12	11.35	10.21	606
24	9.85	19.12	578
37	8.27	28.49	571
54	6.38	39.72	563
72	4.63	50.10	554

Decreasing values of n, take the first.

TABLE VIII. Temperature 39°.

Experiment No. 25 b. $p_{H'} = 8.46$. Borate.

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$
0	13.06	—	—
38	12.83	1.366	26
77	12.58	2.849	26
115	12.32	4.393	27
153	12.10	5.701	26
		Mean	26

Experiment No. 13 b. $p_{H'} = 7.70$.

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$
0	13.07	—	—
142	5.57	44.53	244
190	3.60	56.22	242
250	1.50	68.69	244
295	0.36	75.46	242
		Mean	243

Experiment No. 3 b. $p_{H'} = 7.32$.

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$
0	13.07	—	—
40	9.12	23.45	429
67	6.73	37.58	427
88	5.03	47.74	428
112	3.32	57.89	428
		Mean	428

Experiment No. 6 b. $p_{H'} = 7.28$.

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$
0	13.07	—	—
51	7.86	30.93	453
70	6.16	41.03	451
95	4.13	53.08	451
116	2.66	61.80	451
		Mean	452

Experiment No. 4 b. $p_{H'} = 7.31$.

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$
0	13.10	—	—
40	8.78	25.65	472
56	7.23	34.85	470
85	4.63	50.23	471
110	2.75	61.45	472
		Mean	471

Experiment No. 2 b. $p_{H'} = 6.85$.

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$
0	13.08	—	—
30	7.98	30.28	752
41	6.30	40.25	753
53	4.63	50.18	754
67	2.96	60.08	751
		Mean	753

Experiment No. 5 b. $p_{H'} = 6.62$.

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$
0	13.09	—	—
26	8.12	29.51	844
36	6.40	39.72	847
47	4.70	49.82	843
59	3.05	59.61	844
		Mean	845

Experiment No. 19 b. $p_{H'} = 6.56$.

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$
0	13.07	—	—
24	8.23	28.74	889
34	6.41	39.55	889
46	4.47	51.00	887
60	2.51	62.70	889
		Mean	889

Experiment No. 21 b. $p_H = 6.16$.

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$
0	13.06	—	—
24	7.73	31.64	986
30	6.52	38.84	989
43	4.19	52.66	986
53	2.50	62.70	988
		Mean	987

Experiment No. 9 b. $p_H = 5.30$.

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$
0	13.07	—	—
21	7.93	30.52	1084
29	6.21	40.72	1079
38	4.43	51.30	1081
49	2.56	62.40	1080
		Mean	1081

Experiment No. 1 b. $p_H = 4.69$.

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$
0	13.08	—	—
21	7.89	30.82	1095
28	6.36	39.90	1092
37	4.54	50.71	1094
47	2.79	61.08	1095
		Mean	1094

Experiment No. 18 b. $p_H = 3.65$.

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$
0	13.04	—	—
8	11.21	10.86	968
16	9.46	21.25	968
24	7.78	31.23	972
33	6.08	41.32	964
		Mean	968

Experiment No. 20 b. $p_H = 2.93$.

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10$
0	13.08	—	—
8.5	11.46	9.618	805
18	9.85	19.18	773
27.5	8.28	28.49	768
39	6.53	38.89	762
50	5.05	47.67	752

Decreasing values of n, take the first.

Experiment No. 14 b. $p_H = 5.70$.

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$
0	13.08	—	—
22	7.93	30.58	—
30	6.07	41.62	1069
39	4.36	51.77	1065
49	2.64	61.98	1072
		Mean	1069

Experiment No. 10 b. $p_H = 4.94$.

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$
0	13.07	—	—
21	7.87	30.87	1097
28	6.31	40.14	1100
37	4.51	50.83	1098
47	2.77	61.15	1096
		Mean	1098

Experiment No. 15 b. $p_H = 4.47$.

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$
0	13.07	—	—
22	7.66	32.12	1094
30	5.92	42.45	1093
39	4.18	52.78	1091
49	2.50	62.76	1090
		Mean	1092

Experiment No. 24 b. $p_H = 3.25$.

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$
0	13.05	—	—
9	11.17	11.16	886
18	9.43	21.49	870
27	7.77	31.34	867
37	6.07	41.45	863
48	4.39	51.41	858

Decreasing values of n, take the first.

Experiment No. 22 b. $p_H = 2.39$.

Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10$
0	13.07	—	—
10	11.50	9.322	663
20	10.18	17.16	619
32	8.71	25.89	596
45	7.16	35.10	596
60	5.68	44.35	575

Decreasing values of n, take the first.

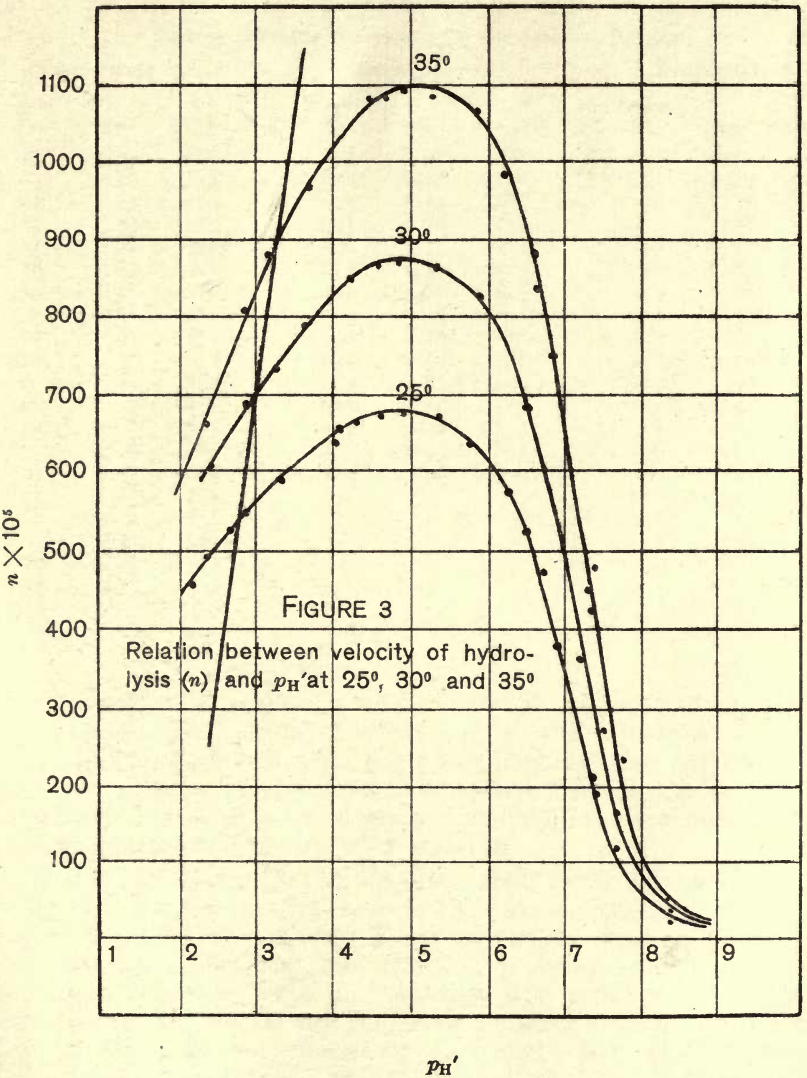
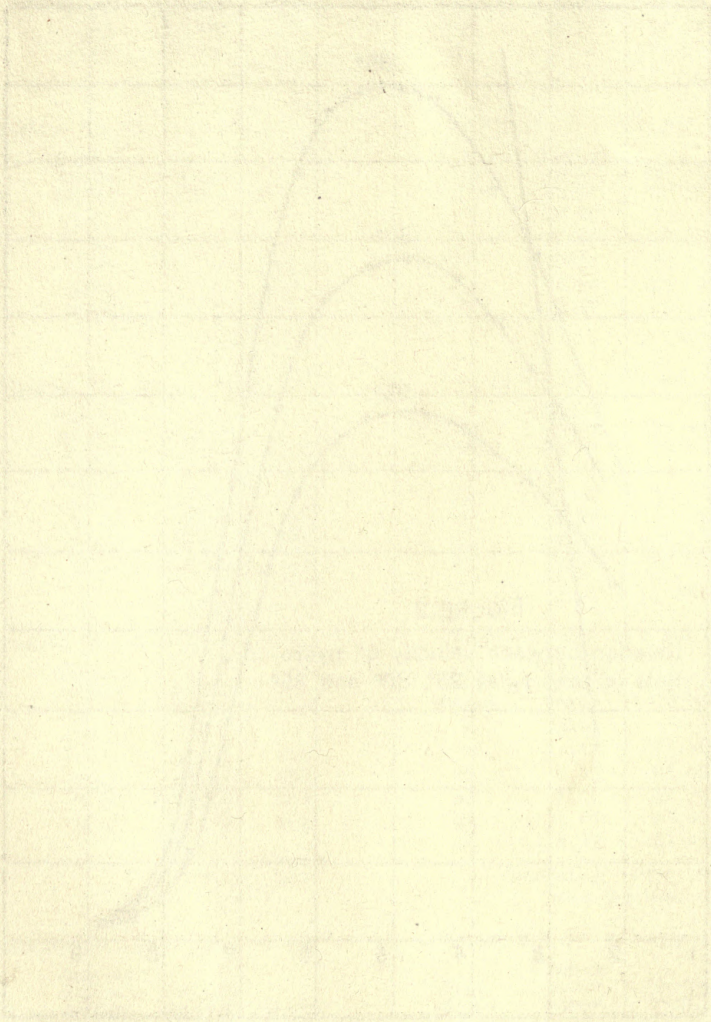


FIGURE 3

Relation between velocity of hydrolysis (n) and p_H' at 25°, 30° and 35°



The results of the experiments are arranged in table IX so as to bring out the relation between the velocity of the reaction (of which n is a masure) and the hydrogen ion concentration of the solution.

TABLE IX.

Relation between the velocity of the reaction and p_H.

Temperature 25°			Temperature 30°			Temperature 35°		
Exper. No.	p_H.	$n \times 10$	Exper. No.	p_H.	$n \times 10^5$	Exper. No.	p_H.	$n \times 10^5$
46	8.43	10	15 a	8.41	18	25 b	8.46	26
45	7.70	113	13 a	7.67	170	13 b	7.70	243
47	7.43	193	12 a	7.45	269	3 b	7.32	428
10	7.33	213	11 a	7.21	364	6 b	7.28	452
11	6.92	375	10 a	6.75	603	4 b	7.31	471
48	6.67	474	14 a	6.52	686	2 b	6.85	753
13	6.48	520	17 a	5.80	825	5 b	6.62	845
8	6.26	572	7 a	5.29	861	19 b	6.56	889
12	5.67	654	25 a	4.88	871	21 b	6.16	987
17	5.32	678	19 a	4.68	871	14 b	5.70	1069
40	4 88	682	21 a	4.27	855	9 b	5.30	1081
41	4.59	681	22 a	3.66	789	10 b	4.94	1098
42	4.27	671	26 a	3.24	737	1 b	4.69	1094
27	4.06	660	23 a	2.93	693	15 b	4.47	1092
44	4.02	654	24 a	2.38	606	18 b	3.65	966
43	3.76	641				24 b	3.25	885
37	3.68	631				20 b	2.93	805
24	3.25	587				22 b	2.39	663
29	2.85	553						
34	2.76	540						
31	2.37	492						
39	2.18	455						

The values of n, which are a measure of the velocity of the hydrolysis, are calculated according to the equation of Nelson and Hitchcock. When they are plotted against the corresponding p_H-values, smooth curves can be drawn through the points thus obtained. These, Figure 3, show the familiar phenomenon of an optimum zone of the enzyme's activity.

Discussion of the curves. Figure 3 shows that the curves extend from the extreme alkaline limit of the enzyme's activity to a p_H of about 2.0—2.5. Beyond the latter points the acid inactivation of the invertase was so great that no reliable measure could be obtained of its activity. The straight line is drawn through the three curves to divide the region of constant n-values on the right from that on the left where the n's show a falling off during the reaction. The constancy of n is thus established (but see above remarks as to the borate buffer) from p_H = approx. 3.0 to p_H = approx. 8.5, which is a considerable extension of the limits for which Nelson and Hitchcock originally applied it (p_H = 4.5

to p_H'= 6.5). It is thus established that invertase acts uniformly between above limits.

The straight line in Figure 3 slopes toward the right with reference to the p_H'-axis. This means that at lower temperatures a greater acidity is necessary (p_H' = 2.75 at 25°) to first bring about inactivation of the invertase with the resultant falling off of n than at higher temperatures where a smaller hydrogen ion concentration suffices to bring about this effect (p_H' = 3.0 at 30° and p_H' = 3.3 at 35°) This result is supported by data of Sörensen (Biochem. Z., **21**, 131 [1909] and Michaelis and Menten. The former, at 52.1°, indicate that invertase is first progressively inactivated at p_H' = approx. 3.90, while the latter, at 22.3°, show this to be the case at p_H' = approx. 2.5.

The data are collected in Table X and plotted in Figure 4 in order to bring out the effect more clearly.

TABLE X.

Relation Between the Temperature and the Hydrogen ion Concentration at which the Inactivation of Invertase During the Hydrolysis First Becomes Noticeable.

Temperature	Critical p_H'
22.3°	2.5
25.0	2.75
30.0	3.00
35.0	3.3
52.1	3.9

The curves of Figure 3 further show that the optimum activity at all three temperatures lies between p_H' = 4.5 and 5.0. The corresponding values obtained by other authors were:

Author	Optimum p_H'
Sörensen	4.0 — 5.0
Michaelis & Davidsohn	3.5 — 5.2
Euler & Emberg	4.2 — 5.2

It is seen that the present method of using n as a criterion of velocity gives the optimum zone as lying within a narrower range than that determined by above workers.

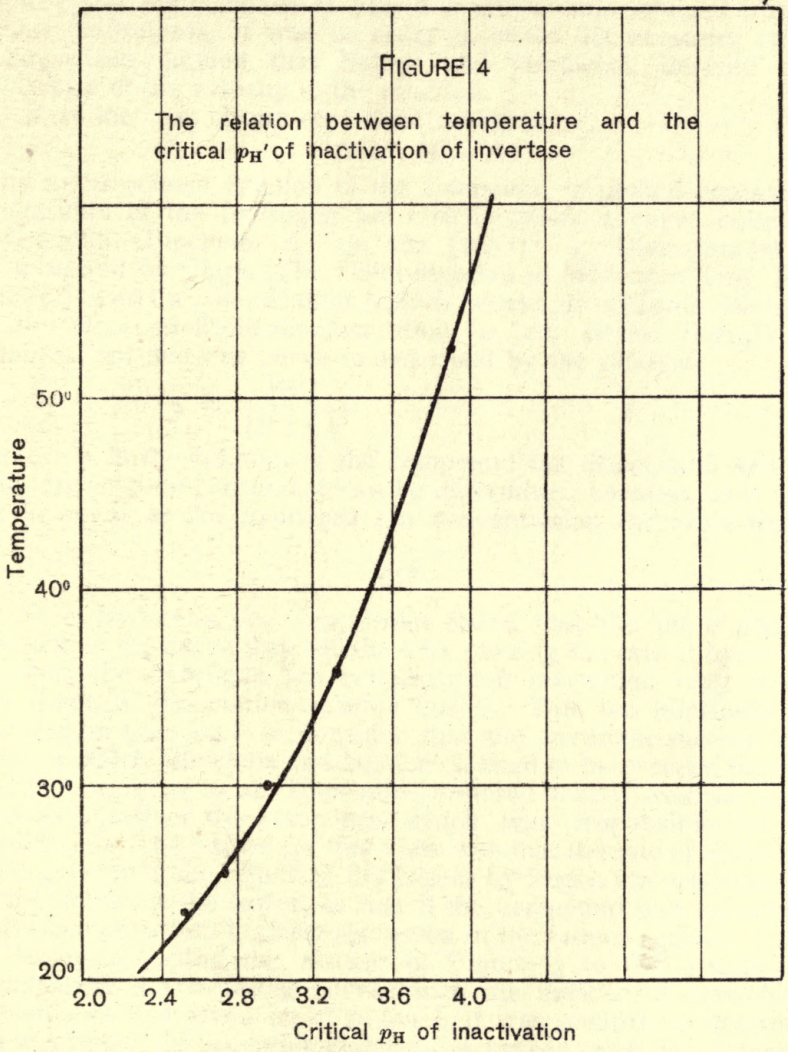
Michaelis and Davidsohn (loc. cit.) were the first to study the shape of the curve obtained by plotting the rate of hydrolysis against the hydrogen ion concentration from a quantitative standpoint. They showed the velocity of hydrolysis to be inversely proportional to the time required for the inversion of any given fraction of the substrate and expressed this by the following relation:

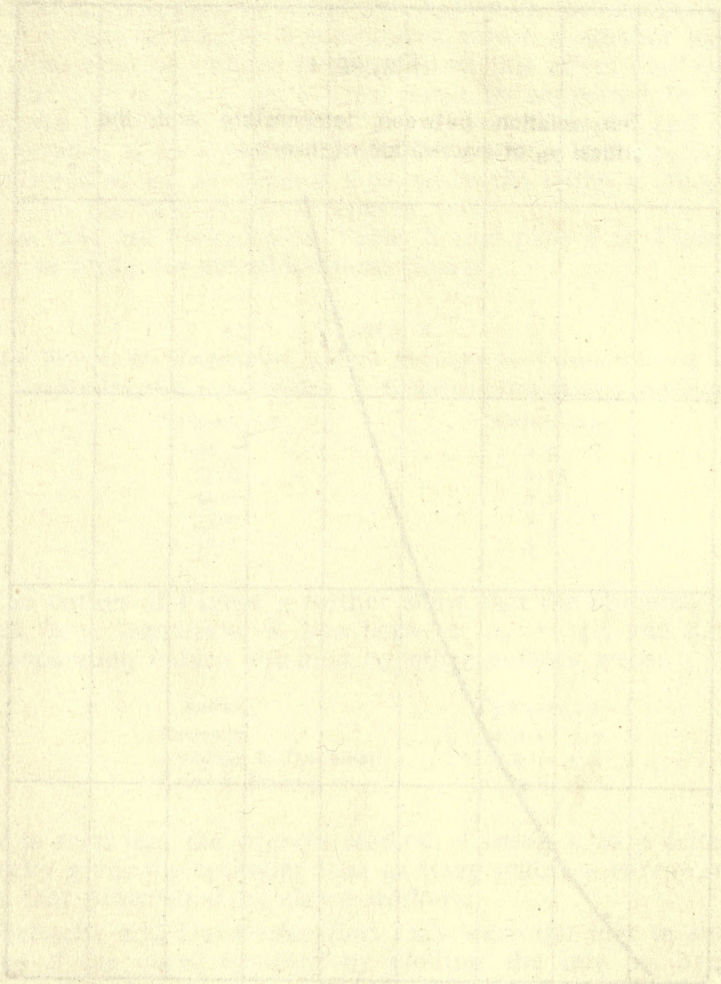
$$\text{Velocity of Hydrolysis} \propto f(a, x)$$

where a is the initial concentration of the substrate and x the fraction inverted in time t. Bayliss (Proc. Roy. Soc., London, **84 B**,

FIGURE 4

The relation between temperature and the critical p_H of inactivation of invertase





90 [1911]) had previously pointed out that this method was least open to objection, so long as no accurate expression for the course of the hydrolysis was available.

Michaelis and Davidsohn's method of determining the velocity of inversion required the plotting of what they termed a standard curve and the comparison with this of the data obtained from all other hydrolyses. It was in order to avoid the necessity of this comparison method that Nelson and Hitchcock derived n as measure of the velocity of the reaction.

If, in the case of any reversible reaction:

$$HA \rightleftarrows H^{\cdot} - A^{-}$$

the undissociated fraction of the compound is plotted against the logarithm of the hydrogen ion concentration, a curve called by Michaelis (Biochem. Z., 33, 182 [1911]) a "Dissoziationsrest" curve, and by Clark (The Determination of Hydrogen Ions, Baltimore [1920]) a „dissociation residue" curve, is obtained. Michaelis (Die Wasserstoffionenkonzentration, p. 22, Berlin [1914]) has pointed out that this curve is described by the relation:

$$\rho = \frac{[H^{\cdot}]}{[H^{\cdot}] + k} \qquad \text{I}$$

where ρ is the fraction of the compound not dissociated and k is the dissociation constant. k can be determined because, as is seen, it is equal to the hydrogen ion concentration corresponding to

$$\rho = \frac{1}{2}$$

Now, Michaelis and Davidsohn found that the more alkaline branch of the curve they obtained by plotting the rate of hydrolysis against the hydrogen ion concentration resembled very closely in shape a dissociation residue curve. This led Michaelis and Rothstein (loc. cit.) to consider that the invertase-sucrose compound which Michaelis and Menten claimed to be present, behaved as if it were an acid. They also proposed that it was the undissociated part of the compound which was responsible for the hydrolysis. The reason for this view was that the rate of hydrolysis was greater in the region of the higher hydrogen ion concentration than in that of the lower, so that if the compound was an acid its dissociation would be most repressed in the former region.

By transforming the n-scale in Figure 3 to the ρ-scale and comparing the resulting curves with the theoretical dissociation residue curves given by equation I, it was possible to determine how closely the experimental results agreed with the theory of Michaelis and Rothstein.

If, from the experimental curves in Figure 3, one determines the k-values by graphically finding the hydrogen ion concentration corresponding to one half the maximum values of n (n, the rate

of hydrolysis being proportional to ρ) and substitutes these in equation I, one finds the theoretical values of ρ corresponding to these dissociation constants and any chosen values of the hydrogen ion concentration. These theoretical values of ρ can then be compared with the corresponding values on the experimental curves which are obtained by changing the n-scale in Figure 3 in such a way that the maximum value of n is in each case reduced to one, while all other n-values are diminished in proportion.

The values of k obtained from Figure 3, as well as a value taken from a curve of Michaelis and Davidsohn, are as follows:

Temperature degrees	$k \times 10^8$
22.3	20 (Michaelis and Davidsohn)
25.0	10
30.0	7.94
35.0	6.76

The constants for transforming the n-scale to the ρ-scale are the reciprocals of 682, 871 and 1098 at 25°, 30°, and 35° respectively, since these numbers gave the maximum values of n at these temperatures.

In Table XI the theoretical values of ρ obtained by above method are arranged alongside of those obtained from the experimental curves by reducing the n-scale. The original values of n are also indicated.

TABLE XI.

Comparison of the theoretical ρ-values with those obtained by reducing the scale of the experimental curves.

pH	25°			30°			35°		
	$n \times 10^5$	ρ		$n \times 10^5$	ρ		$n \times 10^5$	ρ	
		exp.	theor.		exp.	theor.		exp.	theor.
5.00	680	0.996	0.990	869	0.998	0.992	1097	0.999	0.994
5.50	665	0.975	0.969	852	0.978	0.976	1080	0.983	0.979
6.00	618	0.906	0.909	803	0.922	0.926	1027	0.935	0.937
6.50	516	0.757	0.760	695	0.798	0.799	900	0.819	0.824
7.00	341	0.500	0.500	484	0.556	0.557	655	0.596	0.597
7.50	160	0.235	0.240	242	0.278	0.285	342	0.311	0.319
8.00	50	0.073	0.091	79	0.091	0.112	115	0.104	0.129
8.50	10	0.015	0.031	16	0.018	0.038	24	0.022	0.045

The theoretical values of ρ at 25° in Table XI are plotted in Figure 5 and a broken line curve is drawn through them to make

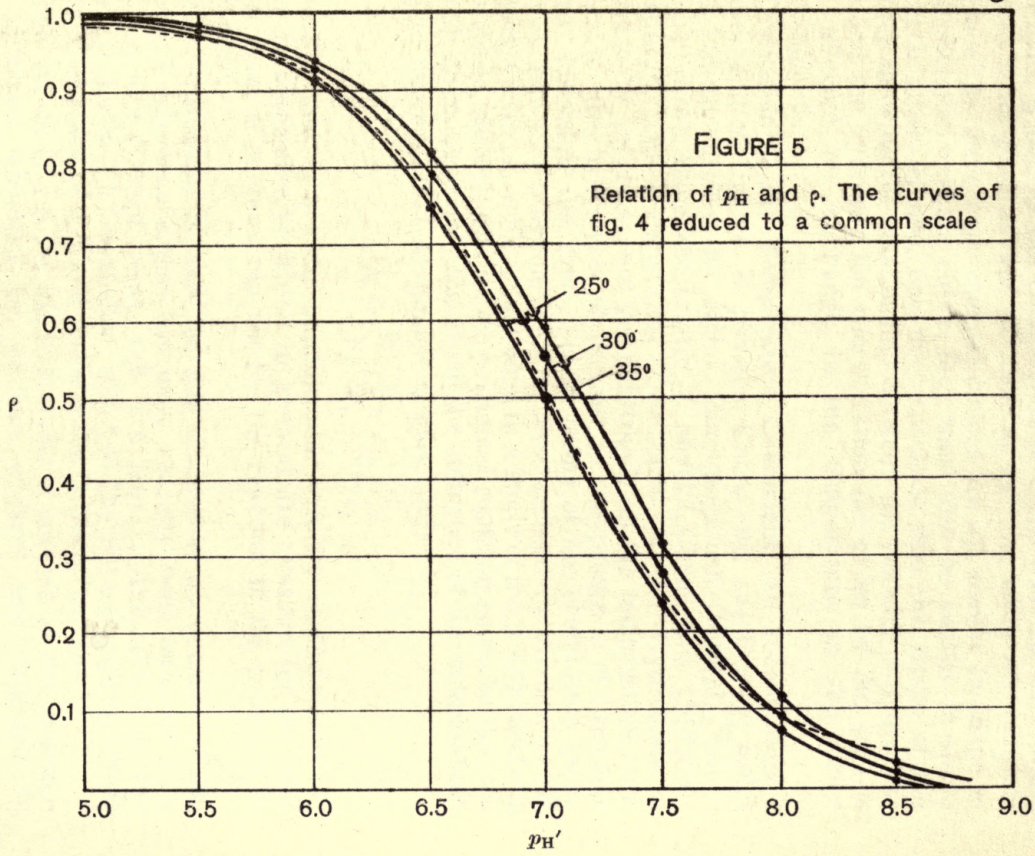


FIGURE 5

Relation of p_H' and ρ. The curves of fig. 4 reduced to a common scale

possible a graphical comparison of the dissociation residue curve thus obtained with the reduced experimental curve. The coincidence is quite close, except near the two ends of the curves, a discrepancy being specially noticeable in the more alkaline region.

The reduced experimental curves at 30° and 35° are also drawn in Figure 5 but, as the coincidence of these with the theoretical curves is as good as was the case at 25°, the latter are omitted.

Since the three experimental curves are all similar in shape to a dissociation residue curve, they must also be similar in shape to each other. Hence, one may conclude that the shape of the curves showing the relationship between the velocity of hydrolysis (n) and the hydrogen ion concentration (as given by the p_H) is not affected by a change in the temperature in the range investigated.

Discussion of the Temperature Coefficient. All recent worker (Euler and af Ugglas, Z. physico. Chem., 65, 124 [1910]; Euler and Laurin, Archiv. för Kemi, Mineralogi och Geologi, Vol. 7, No. 24 [1919]; Euler and Laurin, Z. physiol. Chem., 110, 55 [1920]; Vosburgh, The Temperature Coefficient of the Hydrolysis of Sucrose by Invertase, not yet published) on the temperature coefficient of the hydrolysis of sucrose in the presence of invertase have discussed the same, not in terms of the coefficients themselves, but of a function of these, first derived by Arrhenius (Z. physik. Chem., 4, 226 [1889]) who, in a study of the acid hydrolysis of sucrose, showed that an experimental equation could be derived which quite accurately described the influence of the temperature.

The expression of Arrhenus* is given by:

$$2\,A = \frac{R T_1 T_2}{T_2 - T_1} \log_e \frac{k_2}{k_1}$$

where k_2 and k_1 are the unimolecular velocity coefficients (at T_2 and T_1 respectively) which in general are constant for the acid hydrolysis of sucrose, but not for the reaction in the presence of invertase.

The expression is used in this discussion on account of the physical significance that has more recently been attached to it by Marcelin (Compt. rend., 158, 116 [1914]; Ann. Phys., 3, 120 [1915]), Lewis (J. Chem. Soc., 109, 796 [1916]) and others. Lewis uses E in place of 2 A and terms it the "critical increment".

The values of 2 A for the hydrolysis of sucrose in the presence of invertase obtained by various workers are collected in Table XII.

*) Arrhenius actually used A and not a multiple thereof. 2 A is here considered owing to its identity with E.

TABLE XII.

The Relation Between 2 A and the Temperature.

Author	Temperature interval	Mean temperature	2 A
Euler & Laurin[1]	0.8° — 10.4°	5.6°	11,400
Euler & af Ugglas[2]	0 — 20	10	11,000
Euler & Laurin[1]	0.8 — 18.9	9.9	10,900
Euler & Laurin[3]	10 — 20	15	10,500
Euler & Laurin[1]	10.4 — 18.9	14.7	10,200
Vosburgh[4]	15 — 20	17.5	10,110
Vosburgh[4]	20 — 25	22.5	9,850
Euler & af Ugglas[2]	20 — 30	25	8,040 — 9,340
Tammann[5]	20 — 30	25	7,000
Vosburgh[4]	25 — 30	27.5	8,925
Vosburgh[4]	30 — 35	32.5	8,690
Euler & Laurin[1]	20 — 45.3	32.7	9,200
Kjeldahl[6]	30 — 40	35	8,000
Euler & Laurin[3]	20 — 52.2	36.1	8,800
Euler & Laurin[1]	20 — 52.2	36.1	8,400
O'Sullivan & Tompson[7]	45 — 50	47.5	7,000
Euler & Laurin[1]	45.3 — 52.2	48.8	5,800

1. Z. physiol. Chem., 110, 55 (1920)
2. Z. physiol. Chem., 65, 124 (1910)
3. Arkiv. For Kemi, Mineralogi och Geologi, vol. 7, No. 24 (1919)
4. The Temperature Coefficient of the Hydrolysis of Sucrose by Invertase, not yet published.
5. Z. physiol. Chem., 16, 271 (1892)
6. Medd. Fra Carlsberg Lab., 335 (1881)
7. Trans. Chem. Soc., 57, 834 (1890)

The values of 2 A from the data of Tammann, Kjeldahl, and O'Sullivan and Tompson were calculated by Euler and of Ugglas. The data are plotted in Figure 6.

The results, as a whole, indicate a decrease of 2 A with the temperature. Vosburgh, after plotting the data, concluded that a straight line relation existed between 2 A and the temperature and obtained the following expression relating the two:

$$2A = 12{,}300 - 117\,t$$

which is graphically shown by the straight line in Figure 6.

A similar equation has also been proposed by Euler and Laurin (Z. physiol. Chem., 110, 55 [1920]):

$$2A = 11{,}400\ (1 - 0.009\,t).$$

The data of Table XII were obtained from experiments performed in or near the zone of the optimum activity of invertase. In no case has a systematic investigation been made of the effect of the hydrogen ion concentration, throughout the region of the enzyme's action, on the temperature coefficient of the hydrolysis.

Now, it is known that in and near the optimum zone, the activity of invertase is little affected by changes in the hydrogen ion con-

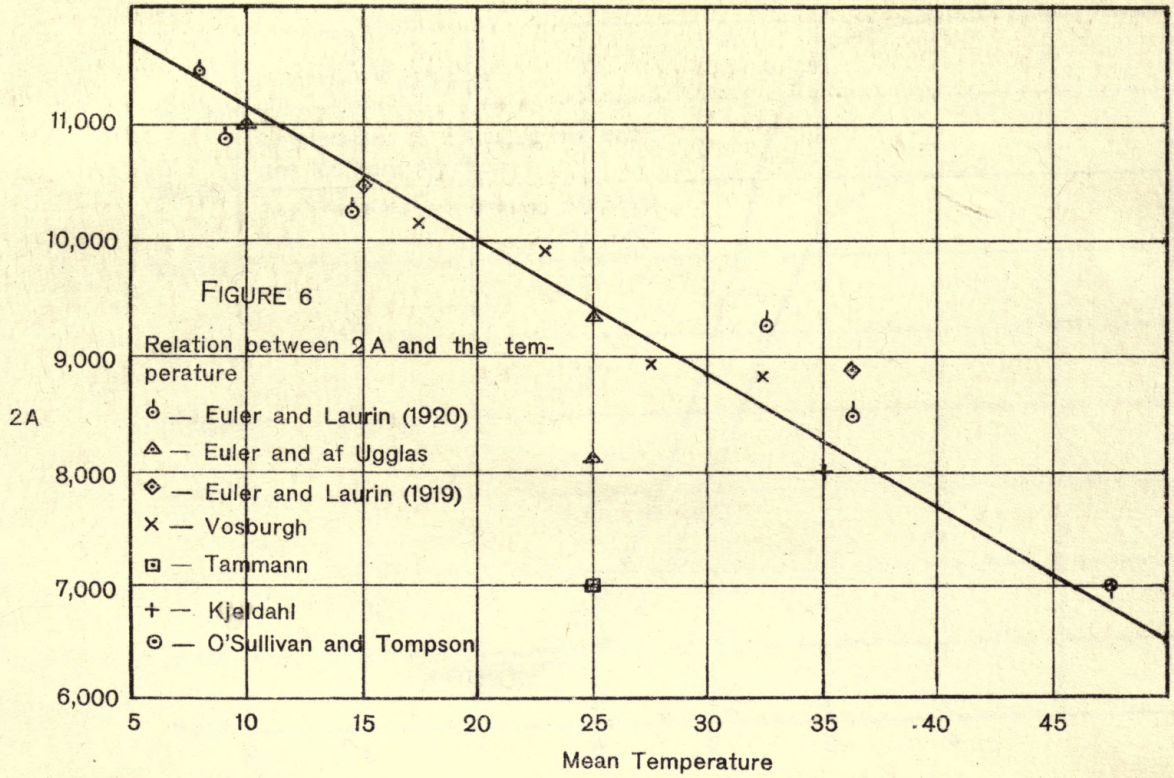
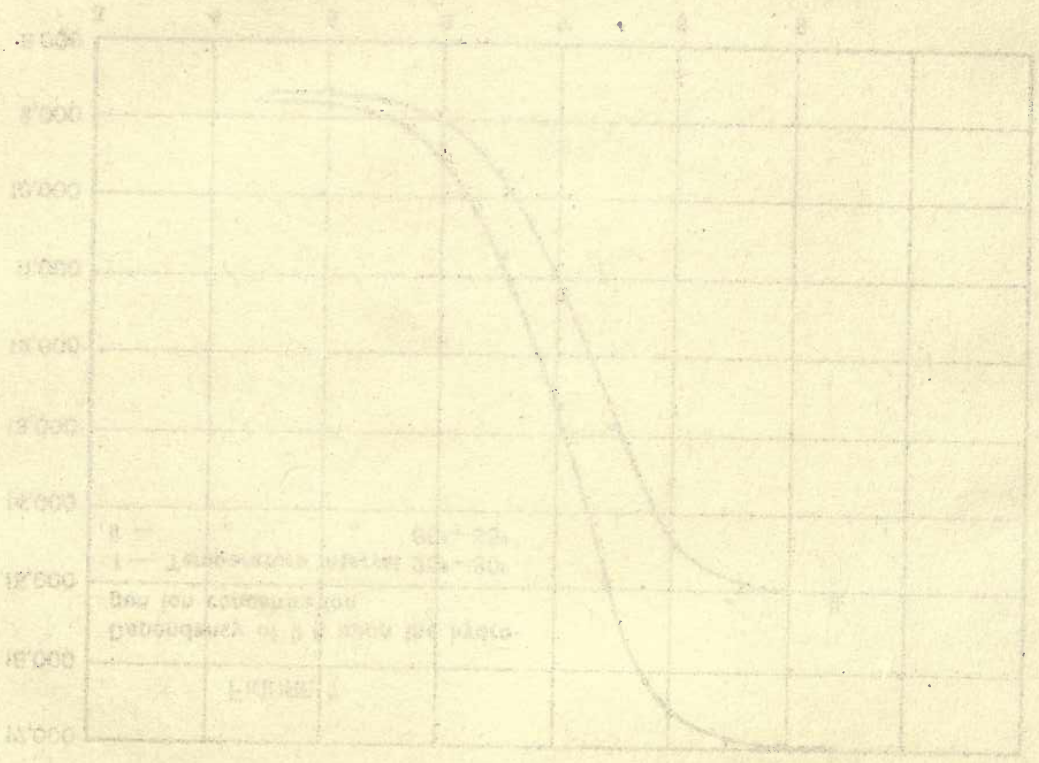

11,000
10,000
9,000
8,000
7,000
6,000
2A
5
10
15
20
25
30
35
40
45
Mean Temperature
Relation between 2A and the temperature
— Euler and Laurin (1920)
— Euler and af Ugglas
— Euler and Laurin (1919)
— Vosburgh
— Tammann
— Kjeldahl
— O'Sullivan and Tompson

FIGURE 6



centration. But if the activities at two temperatures are practically unaffected by changes of the hydrogen ion concentration, then naturally the temperature coefficients, which are the ratios of the activities, will also be but little affected by changes of the hydrogen ion concentration. And since 2 A is a function of the coefficients, it can also be but little changed.

On the other hand, it was thought possible that conditions as to the temperature coefficients might be different in the regions on both sides of the optimum zone where changes in the hydrogen ion concentration have a considerable effect on the velocity of the hydrolysis.

For this reason, the values of n in Table XI (which correspond to the values of k in the expression of Arrhenius) were compared at intervals of hydrogen ion concentration corresponding to 0.5 p_H· throughout the region of the enzyme's activity, and the temperature coefficients and values of 2 A calculated from these.

TABLE XIII.

The temperature coefficients on the more alkaline side of the optimum.

p_H·	$\frac{n_{30^0}}{n_{25^0}}$	$\frac{n_{35^0}}{n_{30^0}}$	E 25^0-30^0	E 30^0-35^0
5.00	1.277	1.263	8,853	8,706
5.5	1.281	1.268	8,945	8,861
6.0	1.299	1.278	9,441	9,181
6.5	1.347	1.295	10,740	9,640
7.0	1.419	1.383	12,630	11,290
7.5	1.512	1.414	14,900	12,900
8.0	1.580	1.455	16,510	14,020
8.5	1.600	1.500	16,960	15,130

It is seen that the coefficients increase with decreasing acidity and decrease with the temperature. The variation of 2 A with the hydrogen ion concentration is shown graphically in Figure 7. The variation is greatest in the region between the optimum zone of the enzyme's activity and the zone of its smallest activity. In these zones it is quite small.

Now, when one examines the curves in Figure 5 which were obtained by reducing the experimental curves of Figure 3 to a common scale, the reason for this dependency of the temperature coefficient on the hydrogen ion concentration becomes evident. If the curves of Figure 3 had all occupied the same position with regard to the p_H·-axis, they would have been reduced to a single curve in Figure 5 and in this case the temperature coefficient of the hydrolysis would have been the same at every hydrogen ion concentration. As it is, however, the curves show a shift along the p_H· -axis with increase in temperature, which is brought out clearly in Figure 5, and it is this shift which accounts for the increase in the temperature coefficients.

Thus, raising the temperature of the hydrolysis seems to have two independent effects, one being an increase in the rate of the reaction and the other a shifting of the curve along the p_H'-axis.

It is interesting to note that while, in the acid hydrolysis of sucrose the temperature coefficient is independent of the temperature, as is indicated by some calculations of Arrhenius from data of Urech (Berichte, 16, 765 [1883]; 17, 2175 [1884]) and Spohr (Z. physik. Chem., 2, 195 [1888]), and of the hydrogen ion concentration (Spohr, loc. cit.; Wilhelmy, Pogg. Ann., 81, 413 [1850]), the coefficient changes with both of these in the hydrolysis of sucrose in presence of invertase.

The temperature coefficients on the more acid side of the optimum zone are given in Table XIV.

TABLE XIV.

The temperature coefficients on the more acid side of the optimum.

p_H'	$\frac{n30^0}{n25^0}$	$\frac{n35^0}{n30^0}$	E 25^0—30^0	E 30^0—35^0
5.0	1.277	1.263	8,853	8,706
4.5	1.276	1.256	8,792	8,507
4.0	1.262	1.247	8,392	8,233
3.5	1.249	1.212	8,011	7,166
3.0	1.237	1.171	7,663	5,895
2.5	1.238	1.104	7,696	3,685

As is seen here, just as in the more alkaline region, the coefficients fall off with increasing acidity. This might on first though be attributed to the faster acid inactivation of the enzyme at the higher temperature than at the lower. A careful study of Figure 3, however, shows that this cannot be the only cause, for the decrease in the coefficients is noticeable not only in the region of decreasing n's, but also where the latter are constant and no inactivation occurs. This means that the temperature coefficients fall off where there is no indication whatever of the enzyme being inactivated.

The phenomenon could be accounted for, just as has been done in the case of the more alkaline branch of the curve, by the assumption that an increase of the temperature not only raises the p_H'-activity curve, but also has the effect of shifting it toward the right. This would cause the coefficients to be smaller where the activities are small (the most acid part of the curves) than where they are large (near the optimum).

The Critical Increment. The interest in Arrhenius' equation has been greatly augmented in recent years by a number of new contributions to the theory of chemical reaction. These theories postulate that only those molecules react which are in an active or "critical state". The fact that they lead to Arrhenius' original equation (in a slightly modified form) may be considered as strong evidence in favor of their validity.

The subject is of special interest in this work owing to the discrepancy in the temperature coefficients in the hydrolysis of cane sugar by acids and by invertase. It is to be hoped that thus in time one or more of these theories may furnish a basis for gaining a closer insight into the nature of the enzyme's action. It is therefore deemed advisable at least to indicate them.

Marcelin (loc. cit.) discusses reaction velocity on a thermodynamic basis and also on some considerations of the theory of probability, and postulates that only those molecules react which reach a "critical" condition, that is, acquire a definite amount of energy in excess of the system's average energy per molecule. Lewis (loc. cit.) has termed this energy difference between the mean state and the critical the "critical increment". Marcelin obtains a constant, E, which is expressed in the same form as Arrhenius' A, but has twice the latter's magnitude. E. has been shown to be practically identical with the critical increment per gram molecule.

Rice (Brit. Assn. Rep., p. 397 [1915]) has extended the results of Marcelin's analysis on the basis of statistical mechanics and by some limiting assumptions has been able to define more closely the critical condition.

Perrin (Ann. Phys., 11, 5 [1919]), Lewis (J. Chem. Soc., 109, 796 [1916]) and Tolman (J. Am. Chem. Soc., 42, 2506 [1920]) considered radiation as the source from which the energy of activation necessary for chemical action was obtained.

Lewis has shown that for homogeneous reactions the temperature coefficient and E, the critical increment, are modified by the catalysing agent. A negative catalyst increases E, a positive catalyst decreases E; and the better (more positive) a catalyst is, the smaller will E be. In support of this idea, Lewis quotes experiments of Halban (Z. physik. Chem., 67, 139 [1909], Bredig and Lichty (Z. Electrochem., 12, 459 [1906]); J. phys. Chem., 11, 225 [1907]), de Bruyn and Sluiter (Proc. K. Akad. Wetensch., Amsterdam, 6, 773 [1904]), and others. In the light of these facts and on the assumption that the hydrolysis of sucrose in the presence of invertase is a homogeneous reaction (a view taken by Michaelis, Biochem. Z., 115, 269 [1921]), the observations in this investigation on the temperature coefficient of the reaction would mean:

1. That invertase at room temperature is a better (more positive) catalyst than hydrogen ion, since in presence of the former E, the energy necessary to change an inactive gram molecule to an active one, is equal to approximately 7,700 — 16,000 calories (depending upon the acidity of the medium) while in the latter E is equal to 26,000 calories. This means that in the presents of invertase less energy is required to convert inactive molecules of the reacting

substances to active molecules than is the case in the catalysis by hydrogen ions.

2. That invertase is a better catalyst in the most acid zone of its activity (p_H = approx. 2.5) where E is smallest, than in the more alkaline regions where E increases as the acidity diminishes.

It must be pointed out, however, that the change of the temperature coefficient and 2 A with the hydrogen ion concentration can equally well be accounted for by the theory of Michaelis and Davidsohn, that the curves relating to the hydrogen-ion concentration and the velocity of hydrolysis are dissociation residue curves of an acid. As previously pointed out, the dependency of the temperature coefficients and 2 A upon the hydrogen ion concentration was shwon in a shift of the curves (Figure 5) along the p_H-axis. According to the theory of Michaelis and Davidsohn these are acid dissociation residue curves and the shift is due to a change in k, the dissociation constant of the acid, with the temperature. That changes in the value of k should have this effect follows from the relationship pointed out in equation I.

EXPERIMENTAL DETAILS.

Preparation of materials. The invertase preparation designated as number 7 by Nelson and Hitchcock was used in all experiments. It was made by previous workers of this laboratory by the method of Nelson and Born (J. Am. Chem. Soc., 36, 393 [1914]) and has been kept several years saturated with toluene in an ice-box. Its activity did not change during the present work as is shown by experiments 40 and 49, which were performed about six months apart.

Experiment 40. November 28, 1921.				Experiment 49. May 17, 1922.			
Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$	Time minutes	Observed rotation degrees	Per cent inverted	$n \times 10^5$
0	13.05	—	—	0	13.07	—	—
33	7.96	30.22	682	333	8.00	30.10	680
45	6.31	40.05	682	45	6.35	39.90	680
59	4.56	50.44	682	59	4.59	50.35	679
75	2.83	60.84	682	75	2.85	60.67	680
		Mean	682			Mean	680

Best commercial (Domino) sugar was used. Its solution in distilled water was stirred up with charcoal, filtered and recrystallized by shaking according to the procedure of Bates and Jackson (Sci. Papers Bur. of Standards, No. 268, p. 75 [1916]). Its rotation agreed within 0.13% with that calculated from the formulas of Landolt and Schönrock (Browne, A Handbook of Sugar Analysis [1912] p. 177-8).

C. P. chemicals were used throughout.

Buffers and solutions during hydrolysis were kept in nonsol bottles.

All volumetric apparatus was calibrated.

Apparatus. The temperature was kept constant in a water thermostat. The fluctuation was $\pm$ 0.01° at 25° and $\pm$ 0.02° at 30° and 35°.

The solutions were placed in 200 mm. polariscope tubes and the rotations read with a Schmidt and Haensch polarimeter reading accurately to 0.01°. Uniform length of the tubes was assured by reading the same 10% sucrose solution through all of them. The temperature of the tubes was kept constant at 25° $\pm$ 0.05° by the thermostat described by Nelson and Beegle (J. Am. Chem. Soc., 41, 559 [1919]).

The light from a mercury vapor arch was passed through two Wratten filters, nos. 77, one of which had been recemented with a green film in place of the yellow. Monochromatic rays of wave length 546.1 $\mu\mu$ were thus obtained. The filters were prepared by Dr. C. E. K. Mees of the Eastman Kodak Company, to whom thanks are due.

Control of the Hydrogen Ion Concentration. The hydrogen ion concentration was kept constant by means of buffer mixtures. The citrate buffers were prepared according to Sörensen (loc. cit.), the borate buffers after Clark, (The Determination of Hydrogen Ions). They were always used in such amounts that their concentration in the solutions undergoing hydrolysis was 0.01 molar. This concentration was so small that the salt effect could be considered practically negligable, as Fales and Nelson (loc. cit.) have pointed out.

Measurement of the Hydrogen Ion Concentration. The hydrogen ion concentration was measured by the electrometric null method. Saturated potassium chloride-calomel electrodes and a satured potassium chloride salt bridge, as recommended by Mudge and Fales (J. Am. Chem. Soc., 42, 2446 [1920]) were used on account of the advantages indicated by these authors. The calomel electrode described by Fales and Vosburgh (J. Am. Chem. Soc., 40 [1918]) and the bubbling hydrogen cell were used throughout the work. The ,plate form of platinum electrode platinized with platinum black was employed. The voltage was determined with a Leeds and Northrup Type K potentiometer and a D'Arsonval galvanometer having a sensitivity of 280 megohms, a period of 2.5 seconds and a total resistance of 444 ohms. A Weston cell was used as a primary standard of reference; it had been rechecked against another standard cell in the department. The electrodes were frequently replatinized and the system was checked at short time intervals with standard 0.1 molar hydrochloric acid to insure accuracy in the experimental determinations.

A sample of this acid was also used for control purposes by Dr. J. C. Morrell, formerly of the Columbia University Chemistry Department, who confirmed its concentration as that indicated above. The compressed hydrogen used was passed successively through neutral permanganate solution, alkaline pyrogallate solution, distilled water (twice) and cotton wool. The rate of flow was 2—3 bubbles per second.

The relationship between E. M. F. and p_H at the different temperatures was obtained from data of Fales and Mudge as to the E. M. F. given by the combination Calomel — Saturated K Cl — Saturated K Cl salt bridge — 0.1 molar H Cl, and of A. A. Noyes (The Electrical Conductivity of Aqueous Solutions, Carnegie Institute, Washington, 1907, p. 141—339) as to the degree of ionization of 0.1 H Cl at different temperatures. Some calculations from the latter paper were obtained from Dr. J. C. Morrell to whom thanks are due.

The relationships obtained are given in Table XV.

TABLE XV.

Degree of Ionization and Potential in Combination with the Calomel-Saturated KCl cell of 0.1 molar HCl.

Temperature	Degree of ionization	Potential volts
25^0	0.9245	0.3100
30^0	0.9251	0.3070
35^0	0.9218	0.3043

With these values and the Nernst equation the following relationships between E. M. F. and p_H were finally obtained:

At 25^0: E. M. F. $= 0.2488 + 0.05911\, p_H$.
30^0: « $= 0.2448 + 0.06010\, p_H$.
35^0: « $= 0.2411 + 0.06110\, p_H$.

Procedure. The procedure followed was in general that recommended by Vosburgh (J. Am. Chem. Soc., 43, 1693 [1921]). The solutions containing buffer and sucrose were made up to volume and such a portion of the latter was taken that when an even number of cc.'s of invertase was pipetted in, the required concentration of each component was obtained. The composition of the solutions undergoing hydrolysis (Tables V, VI and VII) was as follows:

Sucrose	Concentration	10 g. per 100 cc.
Buffer	«	0.01 molar
Invertase	«	5.56 cc. per 100 cc. solution.

The solutions were shaken while being mixed. The pipettes delivered in from 6 to 10 seconds, except the one used for the

invertase whose time was 14 seconds (Simons, Dissertation, Columbia University, 1921, Nelson and Simons, J. Am. Chem. Soc. (to be published later). The mean delivery time of the pipette was taken as the time of observation. 25 cc. samples of the reaction mixture were added to 5 cc. of 0.1 M sodium carbonate solution, which stopped the reaction. Readings were made between 15 minutes and two hours after this, as recommended by Hudson (J. Am. Chem. Soc., 30, 1546 [1908]). For the initial rotation solutions of like composition as the above were made up, but the invertase was inactivated by the sodium carbonate before the sucrose was added. In taking the rotation of the solutions, the polariscope tube was rotated after each reading to avoid errors due to possible strains in the cover glasses. (Browne, A Handbook of Sugar Analysis [1912], p. 156). Four readings were thus taken. The zero point was determined by readings through distilled water.

Calculation of *n*. In calculating the percent inverted in Tables V, VI and VII, the total change in rotation was always taken as 16.84^0 (except where other values are indicated). For justification of this procedure, see Nelson and Hitchcock (loc. cit.) under "Procedure".

Owing to the extreme constancy in the *n*-values during the course of a hydrolysis, it was considered sufficient to make four observations only during an experiment. These, as will be noted in the tables, were in most cases taken at the middle part of the reaction. This was due to the fact pointed out by Nelson and Hitchcock and to be discussed below under the heading "Discussion of Errors", that the experimental errors are the largest in the early part of the inversion. Since, in addition, taking observations toward the latter part of the reaction involves a considerable loss of time, specially if the hydrolysis is slow, it was decided to take readings at the middle part when ever this was possible.

In experiments 46, 34, 31 and 39 of Table V, 15 a, 23 a, and 24 a of Table VI, and 25 b, 24 b, 20 b, and 22 b of Table VII, certain difficulties made this procedure inadvisable. Part of these were on the acid side of the optimum. Here the acidity of the medium was so great as to cause the activity of the invertase to decrease during the reaction, the effect showing up in a falling off of *n*. Since the effect progresses with time, readings in these experiments were taken during the early part of the reaction, before the inactivation of the enzyme could have advanced very far. It was thought this would give the most accurate measure of the activity of the invertase.

In experiments 46, 15 a and 25 b readings had to be made in the early part of the hydrolysis for another reason. Here some unaccounted for effect made itself noticeable after several hours. In every case the first part of the inversion progressed at a rate

to be expected considering the p_H of the medium, and n remained constant. But after this the velocity of the reaction took a sudden jump, the n-values increased correspondingly and the p_H of the solution fell off.

Discussion of Errors. It was desired to find the error in n at any desired stage of the hydrolysis caused by all the separate inaccuracies in measurement that may occur during an experiment, such as in weighing out the sucrose, pipetting the various solutions and taking the times of observation. The calculations were made in collaboration with Mr. Frank Hollander of this laboratory. For n we have:

$$n = \frac{1}{t}\left(\log \frac{100}{100-p} + 0.002642p - 0.0_5886p^2 - 0.0_61034p^3\right).$$

The problem, which is best solved by the method of least squares, is of the type in which the quantity M is a function of a number of directly measured quantities, $m_1, m_2, \ldots\ldots m_n$. It is desired to find the reliability of the result M whose value is computed from the directly measured quantities $m_1, m_2, \ldots m_n$, the precision of measurement of the latter quantities being estimated.

If the precision of $m_1, m_2, \ldots\ldots m_n$, is given by $dm_1, dm_2, \ldots\ldots dm_n$, then the error in M due to the errors in all the directly measured quantities is given by:

$$dM = \sqrt{\left(\frac{\delta M}{\delta m_1} dm_1\right)^2 + \left(\frac{\delta M}{\delta_{m_2}} dm_2\right)^2 + \ldots\ldots\ldots\ldots \left(\frac{\delta M}{\delta m_n} dm_n\right)^2}$$

Changing the notation to apply to the actual problem at hand, we obtain from Nelson and Hitchcock's equation:

$$dn = \sqrt{\left(\frac{\delta n}{\delta t} dt\right)^2 + \left(\frac{\delta n}{\delta p} dp\right)^2}$$

But since 100% hydrolysis is equivalent to a change in rotation of 16.84°, we have

$$dp = \frac{dR_m}{0.1684}$$

and hence,

$$dn = \sqrt{\left(\frac{\delta n}{\delta t} dt\right)^2 + \left(\frac{1}{0.1684} \cdot \frac{\delta n}{\delta p} \cdot dR_m\right)^2} \quad \ldots\ldots \text{II}$$

by partial differentiation of Nelson and Hitchcock's equation, we obtain:

$$\frac{\delta n}{\delta t} dt = -\frac{1}{t^2}\left(\log \frac{100}{100-p} + 0.002642p - 0.0_5886p^2 - 0.0_61034p^3\right)dt \quad \text{III}$$

$$\text{and } \frac{\delta n}{\delta p} dp = \frac{1}{t}\left(\frac{1}{100-p} + 0.002642 - 0.0_41772p - 0.0_63102p^2\right)dp$$

$$= \frac{1}{0.1684\,t}\left(\frac{1}{100-p} + 0.002642 - 0.0_41772p - 0.0_63102p^2\right)dR_m \quad \text{IV}$$

Since in equations III and IV, p stands for the percentage hydrolysed, the values within the parentheses can easily be calculated for various stages of the reaction. In III and IV, dt and dR_m are the precision in the time and the rotation measurements respectively.

Determination of dR_m. The rotation of a solution depends on its composition; the error in rotation (dR_m) on the errors in measurements which are involved in its preparation. In the experiments of Tables V, VI and VII, the sucrose concentration of the solutions in the polariscope tubes was calculated as follows:

Solution A. u = g Sucrose (should be 21.1764) } Sucrose concentration $= \frac{u}{v}$
v = cc. sol'n (" " 200) }

Solution B. w = cc. sol'n A (" " 170) } Sucrose concentration $= \frac{w}{w+x} \cdot \frac{u}{v}$
x = cc. invertase (should be 10) } Invertase concentration $= \frac{x}{w+x}$

Sample. y = cc. sol'n B (" " 25) } Sucrose concentration $= \frac{y}{y+z} \cdot \frac{w}{w+x} \cdot \frac{u}{v}$
z = cc. Na_2CO_3 (" " 5) } Invertase concentration $= \frac{y}{y+z} \cdot \frac{x}{w+x}$

The concentration of the sucrose in the final sample which is read in the polariscope hence is,

$$\frac{y}{y+z} \cdot \frac{w}{w+x} \cdot \frac{u}{v}$$

while the concentration of the invertase in the same sample is

$$\left(\frac{y}{y+z}\right)\left(\frac{x}{w+x}\right)$$

Since a 10% sucrose solution gives a rotation of 15.66°, one of the above concentration will give a rotation of

$$r_s = 15.66 \times 10\left(\frac{y}{y+z}\right)\left(\frac{w}{w+x}\right)\left(\frac{u}{v}\right)$$

This is the effect due to the sucrose alone at the beginning of the hydrolysis when none has been inverted, i.e., time, $t = 0$ and degree of hydrolysis, $\alpha = 0$.

During the hydrolysis, however, the amount of sucrose decreases and invert sugar is formed. Let

r_{IS} = the rotation of an invert sugar solution resulting from the

complete hydrolysis of a cane sugar solution whose concentration is

$$\left(\frac{y}{y+z}\right)\left(\frac{w}{w+x}\right)\left(\frac{u}{v}\right)$$

(see above). Since the final rotation of a completely hydrolyzed 10% sucrose solution (which is considered due to a practically 10% invert sugar solution, since the hydrolysis may be assumed to be complete) is equal to -3.84^0 (calculated from the observed final rotation, -3.79^0 and the known rotation of the invertase present, 0.05^0), we obtain for the rotation of the invert sugar solution from the complete inversion of above sucrose solution:

$$r_{Is} = -38.4\left(\frac{y}{y+z}\right)\left(\frac{w}{w+x}\right)\left(\frac{u}{v}\right)$$

Finally the rotation of the invertase which is optically active must be considered. It was calculated from some data of Dr. D. I. Hitchcock, formerly of this laboratory who found that 0.053 cc. of invertase No. 7 per 1 cc. of solution give a rotation of 0.045^0. Hence the rotation, r_{Inv}, due to an invertase concentration $\left(\frac{x}{w+x}\right)\left(\frac{y}{y+z}\right)$ (see above) is:

$$r_{inv} = \frac{0.045}{0.053}\left(\frac{x}{w+x}\right)\left(\frac{y}{y+z}\right)$$

$$\text{or} \quad r_{inv} = 0.849\left(\frac{x}{w+x}\right)\left(\frac{y}{y+z}\right)$$

If now α = fraction of sucrose solution hydrolyzed, and R_m = the rotation of the mixture at any stage of the hydrolysis, we obtain:

$$R_m = (1-\alpha)\,r_s + \alpha\, r_{Is} + r_{Inv}$$

$$= (156.6)(1-\alpha)\left(\frac{y}{y+z}\right)\left(\frac{w}{w+x}\right)\left(\frac{u}{v}\right) - 38.4\,\alpha\left(\frac{y}{y+z}\right)\left(\frac{w}{w+x}\right)\left(\frac{u}{v}\right) + (0.849)\left(\frac{y}{y+z}\right)\left(\frac{x}{w+x}\right)$$

$$\text{or} \quad R_m = \left[(156.6)(1-\alpha) - 38.4\,\alpha\right]\left[\left(\frac{y}{y+z}\right)\left(\frac{w}{w+u}\right)\left(\frac{u}{v}\right)\right] + 0.849\left(\frac{y}{y+z}\right)\left(\frac{x}{w+x}\right)\Big] \quad \text{V}$$

Now our object is to find the effect on the observed rotation (R_m) of various errors in measuring u, v, w, x, y and z. Similarly as before (equation II) we obtain:

$$dR_m = \sqrt{\left(\frac{\delta R_m}{\delta u}du\right)^2 + \ldots + \left(\frac{\delta R_m}{\delta z}dz\right)^2}$$

The values under the square root sign are obtained from V by partial differentiation. Since R_m depends upon the stage of the hydrolysis (the expression contains the term α), this will also be the case for dR_m. The only thing which remains to be done now, is to estimate the values of $du, dv, \ldots . dz$, the errors in $u, v, \ldots . z$. The following values were thought to be a good measure of these errors:

$du = 0.0005$ g.
$dv = 0.01$ cc.
$dw = 0.05$ cc.
$dx = 0.01$ cc.
$dy = 0.02$ cc.
$dz = 0.005$ cc.

When these and the values for $u, v, \ldots . z$, are substituted in the expression for dR_m, we obtain the following values for the latter at different stages of he hydrolysis:

α	dR_m	α	dR_m
0.1	0.0026	0.6	0.0008
0.2	0.0022	0.7	0.0004
0.3	0.0019	0.8	0.00004
0.4	0.0015	0.9	0.0004
0.5	0.0011		

dR_m is seen to be greatest at the beginning of the hydrolysis, to decrease and to reach a minimum at $= 0.8$. The maximum value of dR_m due to the errors discussed is smaller than 0.003^0. Now the error in reading the polariscope is assumed to be 0.005^0, which is considerably greater than the above values, and hence 0.005^0 must be substituted throughout in IV for dR_m instead of the smaller changing values indicated above.

Final Calculations. dR_m being thus determined, let us assume dt (the error in time readings) $= 2$ seconds. There now remain in III and IV only the values in the parenthesis and the values of t to be determined. The former can be directly calculated for the various values of p corresponding to $\alpha = 0.1, 0.2, \ldots . 0.9$.

For t, the times of the fastest experiment performed, namely that at 35^0 and the optimum hydrogen ion concentration, were used. The object of this was to obtain the largest errors that were liable to occur in the entire experimental work, and thus obtain a limiting value for the error. It is clear that the faster the hydrolysis, the greater the errors; for the smaller t, the greater are the values of III and IV.

In the fastest hydrolysis, the time for 10% inversion was approximately 7 minutes. The times for 20%, 30% . . . . . 90% were then calculated from Nelson and Hitchcock's equation on basis of the known value of n. From these then the corresponding values of $\frac{I}{t}$ and $\frac{I}{t^2}$ were obtained. With the values of the latter, of dR_m,

of dt and of the terms in the parenthesis in III and IV, we can then solve for $\frac{\delta n}{\delta t} dt$ and $\frac{\delta n}{\delta p} dp$, and with these finally for dn.

The latter are given in Table XVI and plotted in Figure 8.

TABLE XVI.

Change of dn (error in n) with the stage of the hydrolysis.

α	$dn \times 10^5$	α	$dn \times 10^5$
0.1	7.51	0.6	1.68
0.2	3.86	0.7	1.69
0.3	2.67	0.8	1.91
0.4	2.11	0.9	2.75
0.5	1.81		

The error is largest at the beginning of the reaction, reaches a minimum near $\alpha = 0.6 - 0.7$ and then again increases. In the actual experimental work the readings were taken near the middle of the inversion. Figure 8 shows more clearly than the table that the errors here are of a small order.

Errors in EMF readings. In a number of sets of parallel experiments it was found that the greatest divergence in EMF values was 0.6 millivolts, and to this corresponded a difference of 0.01 p_H. Since the position of the curves in Figure 5 corresponded to differences of 0.1 and 0.07 p_H respectively, there is no danger of the results being due to experimental errors.

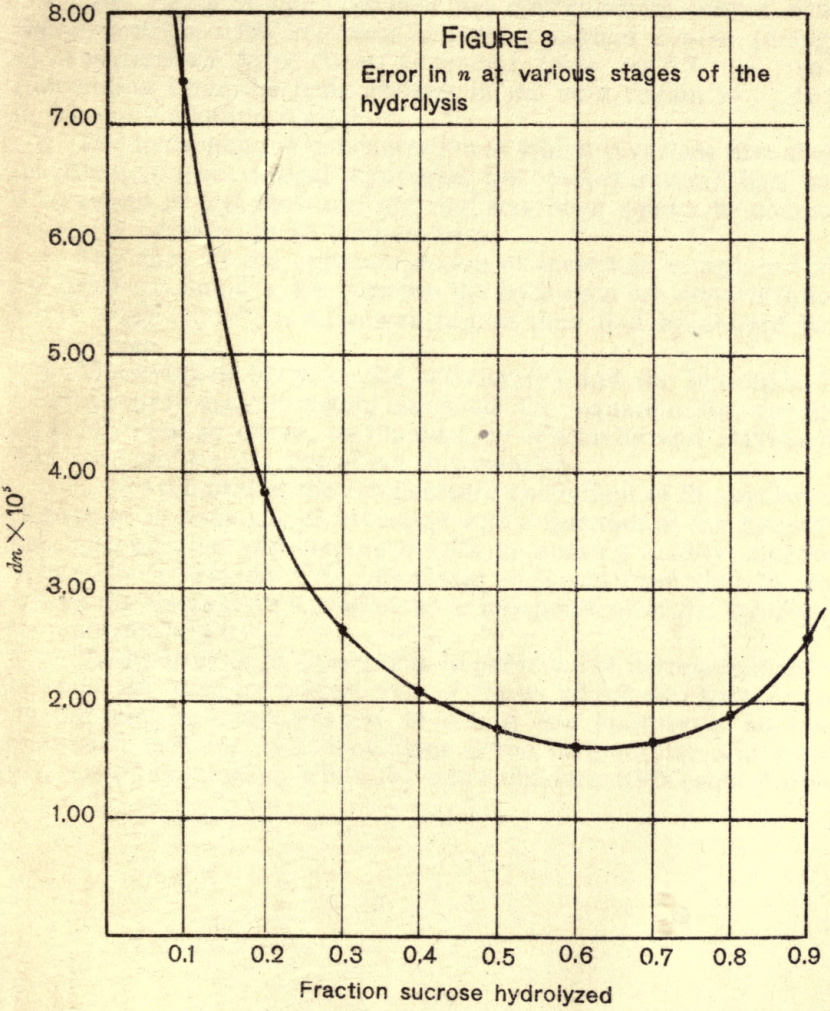
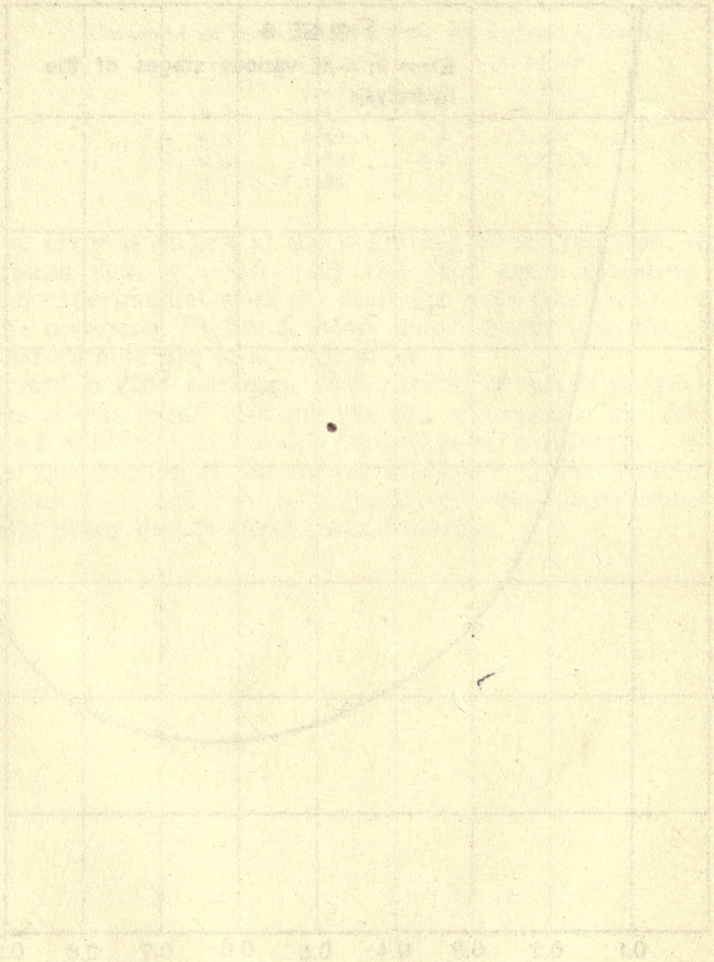


FIGURE 8

Error in n at various stages of the hydrolysis



Summary.

1. It has been shown that the sucrose concentration at which the hydrolysis in presence of invertase attains a maximum velocity is independent of the temperature and the hydrogen ion concentration and that the effect of these latter upon the rate of the reaction is independent of the sucrose concentration.

2. The limits of the hydrogen ion concentration within which the hydrolysis with invertase follows a normal course (using n as a criterion) have been extended from $10^{-2.75}$ — $10^{-3.3}$ (depending upon the temperature) in the acid region to $10^{-8.4}$ in the more alkaline region.

3. The hydrogen ion concentration at which invertase first shows inactivetion (the critical hydrogen ion concentration) has been determined at 25°, 30° and 35° and has been shown to decrease regularly between these temperatures.

4. The zone of the optimum action of invertase at 25°, 30° and 35° has been found to lie between the hydrogen ion concentrations $10^{-4.5}$ and $10^{-5.0}$, a narrower region than had heretofore been determined.

5. The relation between the activity (n) and the hydrogen ion concentration approximately satisfied the equation for the dissociation residue curve, as claimed by Michaelis and Davidsohn. The temperature did not affect this relation.

6. It was found that the temperature coefficient of the hydrolysis of sucrose in presence of invertase was a function of the hydrogen ion concentration and increased with decreasing acidity, and that, hence the hydrolysis was inherently different from that by acid where the temperature coefficient is independent of the hydrogen-ion concentration.

7. The hydrolysis of cane sugar in presence of invertase involves at least two distinct stages. One of these, which is characterized by the sucrose concentration at which the hydrolysis attains a maximum velocity, is independent of the temperature and hydrogen ion concentration, while the other changes with each of these.

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